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ASSESSMENT OF OPTIMUM AQUATIC MICROCOSM DESIGN FOR POLLUTION IMPACT STUDIES

Author
Harte, J.

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UNIVERSITY OF CALIFORNIA

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John Harte, Donald Levy, John Rees,
and Ellen Saegebarth

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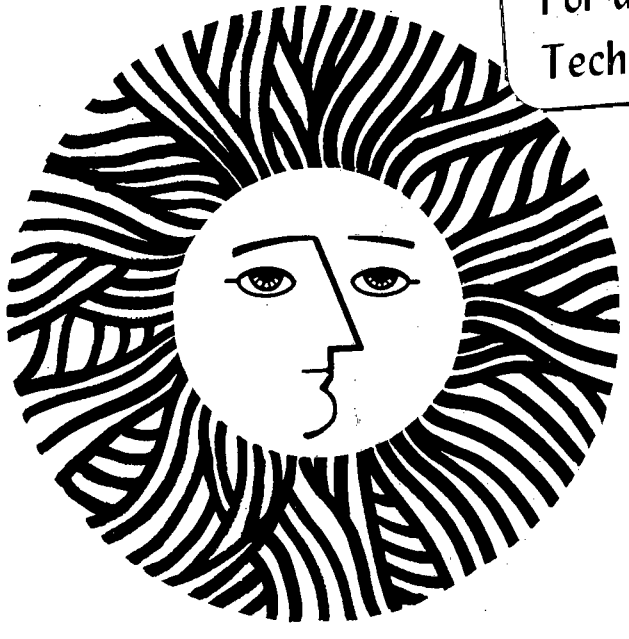

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ASSESSMENT OF OPTIMUM AQUATIC MICROCOSM DESIGN FOR POLLUTION IMPACT STUDIES

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Prepared by

LAWRENCE BERKELEY LABORATORY
University of California
Berkeley, California 94720

Investigators

John Harte
Donald Levy
John Rees
Ellen Saegebarth

Prepared for
Electric Power Research Institute
3412 Hillview Avenue
Palo Alto, California 94304

EPRI Project Manager
Robert Brocksen
Energy Analysis and Environment Division

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ABSTRACT

A series of experiments was carried out to determine and evaluate optimum design and operating conditions for pelagic lake microcosms (microcosm assessment studies) and to explore a possible use of such systems for toxicological testing (decomposition studies). Criteria selected for microcosm optimization were realism (tracking by the microcosms of the real lake used to stock the microcosms) and replicability of identically-initiated microcosms. In the assessment studies, a number of different pelagic microcosm configurations were studied, including the size of the microcosm containers (from 4 liters to 200 liters), the method of algal surface-growth prevention, and the degree of water mixing and aeration. In addition, the microcosm-lake comparisons were carried out at various seasons of the year, allowing us to determine the influence of natural seasonal factors on the chemical and biological differences between the lakes and the microcosms.

In all but the smallest microcosms, surface-growth prevention removed size-dependence. Chemical nutrients tracked well except during periods when nutrient inputs to the lake from the surrounding watershed were high. Good tracking of phytoplankton succession patterns was observed only when the physical conditions of the lake matched well those in the laboratory system. In the decomposition studies, additions of dead organic matter to the lake microcosms were made and the subsequent response of mineralization activity measured. Highly replicable and interesting short-term behavior was seen, implying that protocols can be developed for microcosm testing of effects of toxicants on mineralization rates. On the basis of the microcosm assessment and decomposition studies, we conclude that appropriate applications of pelagic microcosms are limited and we delineate those applications that are most appropriate.

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EXECUTIVE SUMMARY

FOCUS OF RESEARCH

Ecological microcosms are segments of natural ecosystems. Their small size permits their use in the laboratory in numbers sufficiently large to allow careful statistical analysis of their behavior. Microcosms can be set up in such a manner as to contain, at least initially, a great deal of the complexity of natural ecosystems, and thus ecosystem-level properties such as nutrient cycles, and not just single-species behavior, can be studied. Moreover, in order to analyze environmental impacts of human activities, microcosms can be polluted or otherwise disturbed at relatively little expense and without the necessity of damaging natural systems.

Recent burgeoning interest in microcosms as a tool for environmental impact assessment and prediction stems from a perception of these advantages, along with a growing awareness of the inadequacy of traditional field-testing and single-species testing approaches. The hypothetical example of a coal-fired synthetic fuel production plant discharging treated waste water into a lake serves to highlight this point. The untreated liquid waste from such a plant is likely to contain at least 50 elements and hundreds or thousands of organic compounds. Many of these, such as cadmium, nickel, phenols, and 3,4-benzo(α)pyrene, are known toxicants. The choice of water-treatment scheme will determine the proportions and amounts of the residual constituent toxicants in the discharge water. Determining the appropriate method and level of waste-water treatment will require considerable understanding of the effects of the constituent toxicants singly and in various combinations, both on aquatic processes such as nutrient cycling, which indirectly influence aquatic biota, and directly on aquatic organisms.

The task of determining an economically optimum water-treatment strategy by carrying out field tests of the ecological effects of the various possible treatment-plant products would be staggering. Many systems of the type one was

trying to protect would have to be degraded in the process, and the costs would be enormous. Statistical confidence in the results would be lacking. To avoid this, a rapid, cheap, reliable, and non-destructive test method is required.

Whether microcosms will be of major use in this context depends to a great extent on how they are designed and operated. It is most essential that their application be matched to their characteristics, as the potential for misuse is considerable. The focus of the microcosm research program at the Lawrence Berkeley Laboratory is the determination of lake microcosm characteristics, the optimization of lake microcosm design and operation, and the identification of appropriate uses for microcosms in environmental impact assessment. The boundaries of this problem are large, as can be seen from a partial list of the design and operation options available for lake microcosms:

- The physical environment of the microcosm contents--container size, shape, and material; light and temperature levels.
- Method of initiation--whole-water-sample from a specific lake versus "cookbook" addition of selected organisms from a variety of sources into a standard nutrient broth.
- Segment of lake modeled--the isolated water column (or planktonic community) versus the lake-bottom sediments plus the water column; inclusion or exclusion of fish and large aquatic plants.
- Hydraulic properties--the rate at which water is stirred or agitated in the microcosm; chemostat conditions with hydraulic flow-through versus batch-culture conditions.
- Surface-growth control--ignoring the problem and letting surface growth of algae and bacteria go unchecked (thus creating large uncertainties in the interpretation of toxicology data due to the distorting influence of surface growth in laboratory systems with surface-to-volume ratios that are considerably larger than in natural lakes) versus biological control of surface growth versus the use of a pouring or siphoning strategy to prevent such growth.

In order to decide among the options listed above and others as well, a set of criteria are needed. We have selected three such criteria: replicability, realism, and simplicity; because the choice of criteria has strongly influenced the direction of our research, we motivate our choice below.

CRITERIA FOR OPTIMIZATION

Replicability: One of the potential advantages of microcosm studies relative to field investigations is that microcosms can be set up in replicate so as to

allow a careful statistical comparison of control and treatment systems. This advantage will not materialize if microcosms are designed and operated in such a fashion that initially identical systems diverge in time. Even under the most careful operating procedures this can happen simply because of the stochastic aspects of organism distribution and behavior. For example, organisms present in low densities in the field system that is the source of the microcosm materials are not likely to be evenly distributed initially among replicate systems. For this reason, we place a great deal of emphasis on measuring and quantifying the degree of replication in aquatic microcosms and the dependence of that replicability on the choice of design and operating procedures.

Realism: We wish to enhance the likelihood that the results of a toxicology test carried out in a lake microcosm will be applicable to a particular natural lake or will be generalizable to a class of lakes. Microcosms are most likely to be realistic, in the above sense, if they behave as similarly as possible to natural systems. Although one might hope to develop mathematical models to extrapolate to a field context the results from microcosms that behave quite differently from the field, it is clearly preferable to start out with microcosms that behave as closely as possible to the natural world. If unpolluted, or control, lake microcosms behave quite differently from a natural lake, then it is highly unlikely that the behavior seen in polluted, or treated, microcosms will have predictive value for that lake. We have adopted the viewpoint that realistic behavior is most likely to be obtained, in control and in treatment systems, if microcosms are initiated directly from whole portions of natural systems rather than by cookbook procedures. The succession patterns of the biota and the fluxes of nutrients, will not be realistic if the components of the microcosms are assembled from diverse sources. However, simply taking a whole-water sample from a lake into the laboratory does not guarantee that the microcosm so initiated will evolve in time in a manner similar to the lake. Much of our research reported here is directed toward characterizing the length of time during which microcosms do behave realistically and understanding how the lake-microcosm similarity can be enhanced and prolonged.

An important issue in this regard is the degree of realism necessary for microcosms to be of use in ecotoxicology. Suppose a microcosm is designed to be a model of the epilimnion of a large stratified lake. We can then ask: of which

microcosm and lake variables should we require similar behavior? What degree of similarity should we require? For how long should this tracking be maintained? The choice of variables and the required duration of tracking can only be determined by the type of toxicological application anticipated. A study of the direct effects of a pollutant on microbial mineralization of a rapidly-cycling plant nutrient could be carried out in a few weeks, and thus long-term realistic behavior would not be required. However, if one wanted to study genetic adaptation of the microbes to the pollutant, longer-term realism would be necessary. Often the bloom and crash of a phytoplankton population in a lake, and associated successional changes in the dominant species of phytoplankton, take place within a few months. Thus tracking of these variables might be required over several months for studies on direct pollutant effects on algal growth and succession.

A more difficult question is the degree of tracking required. Can a microcosm result be considered valid for the parent lake from which the microcosm was derived if the control microcosm variables differ from the lake variables by, say, 30%. Linked to this issue is the problem of determining how similar the behavior must be within a set of replicate microcosms. A detailed treatment of these topics is contained within this report. We provide a practical prescription for the appropriate statistical measures to use in analyzing the degree of tracking of lakes and microcosms and the degree of replication of microcosms, along with a description of appropriate time-averaging procedures. The latter is particularly important in order to avoid being misled by small phase-differences between the time-dependence of variables in lakes and in microcosms or among replicate microcosms.

Simplicity: The third criterion is simplicity. Microcosms that are expensive to construct, initiate, and operate, that require large numbers of highly-trained personnel to monitor and maintain, and that are of such complex mechanical structure that breakdowns occur, are not likely to be of widespread use in environmental impact assessment. In Section 2 we describe a number of simplifed design and operating procedures that we employ for lake microcosms. The extent to which the adoption of these simplifications either diminishes or enhances replication and realism is discussed both there and in Section 5.

Our approach to surface-growth prevention involves periodic transfer of the microcosm contents to clean containers. This approach is particularly simple,

and yet more effective that other more complicated approaches such as biological control. Our strategy for initiation of the microcosms, by simply removing to the laboratory whole water samples from lakes, is far simpler than is the "cookbook" approach in which selected organisms are assembled from diverse sources and added to an artificial nutrient medium. Moreover, it enhances the realism of the microcosms and increases the chances that the results of toxicology studies carried out in such systems will be applicable to natural systems.

The level of training needed to operate microcosms effectively depends on the stage of development and application. In the early stages, in which optimum microcosms are being developed, it is critical that skilled taxonomists are available in order to determine the species composition and assess the degree to which the biota in the microcosms track those in the parent system. As explained in more detail in Section 1, microcosms in which only general chemical relationships resemble those in the field, but which have markedly different species composition over time, are not likely to provide realistic information about ecological effects of pollutants. However, once suitable prescriptions are available for operating microcosms with realistic chemical and taxonomic parameters, then for certain applications regular taxonomic monitoring will not be necessary.

METHODS AND RESULTS

In order to optimize lake microcosms for environmental impact assessment, we carried out a number of experiments that are described within this report. In the first four of these experiments, which formed the core of the research project, we compared a variety of microcosm designs and operating conditions in order to understand their influence on microcosm behavior and the degree to which they either enhance or reduce realism and replicability. The conditions which were varied in these experiments included the size and shape of the microcosm containers, the degree of aeration and water agitation in the microcosms, the degree of surface growth of algae on the inner walls of the containers, and the method of preventing that growth. Three of the four experiments were what we call "tracking studies," in which microcosms set up initially with a water sample taken from a particular lake are then compared on a weekly basis with the parent lake. These tracking studies were carried out on two different stratified lakes, and at two different times of the year in

which the behavior of the plankton in the lakes was markedly different. Only the surface waters of the lakes were used to stock the microcosms initially and only the surface waters were sampled weekly. Thus the microcosms that were tested here were models of the pelagic communities in the lake epilimnion and not models of the benthic zone and its immediately overlying water. Extension of our work to benthic microcosms is now in progress.

The results of these studies demonstrate both the dependence of replicability and realism on design and operating conditions and the feasibility of pelagic microcosms for certain types of toxicological testing. Surface-growth prevention generally enhances the value of microcosms in this context. Although very small microcosms of shallow depth behave unrealistically and in a manner that is size-dependent, these problems are not present in the microcosms we studied that were greater than 15 liters in volume and 70 cm in depth. With volume-dependence of microcosm behavior absent above critical volume, the potential exists for wider flexibility and generalizability of microcosm results in ecotoxicology. Further work on depth-dependence is needed, however, to complete this analysis.

In a fifth experiment, we examined the behavior of microcosms over a two-year period, in contrast to the first four experiments, which looked at lake microcosms over periods of only two to three months with applications to relatively short-term impact testing in mind. Microcosms of two different sizes, both with and without surface growth, were compared in this experiment. The persistence of population cycles and successional changes, along with enhanced replicability of the systems without surface growth, were observed over the two-year period.

The final experiment explored a possible application of microcosms. It consisted of four sub-experiments in which lake water housed in microcosms was subjected to increases in organic matter. This basic strategy was repeated four times in order to assess the universality of the observed phenomena and to determine how the responses of lake water to organic matter loading depend on experimental conditions. When subjected to increased food supply in the form of killed organic matter, the microorganisms in lake water that are responsible for decomposition and production of inorganic nutrients such as ammonia begin to increase in numbers and in activity. From the detailed pattern of response,

particularly by noting the dependence of inorganic nitrogen production on the amount of organic matter added, we learned useful information about the population dynamics and the activity of the microorganisms. These studies suggest a way to develop a simple, rapid, replicable, testing procedure to determine the effects of toxic substances on nutrient cycling in lake waters. Development of such a test protocol and its extension to benthic communities is intended in future work.

Section 1

INTRODUCTION

PURPOSE

Laboratory microcosms may play an important role as experimental tools for examining ecological impacts resulting from human activities. The major advantage of microcosm studies over field research is that the former allow a greater opportunity for manipulation and control. In addition, the expense and environmental risk entailed by microcosm investigations are relatively small compared with field studies of toxic substance effects. Microcosms also potentially offer greater realism than that provided by studies of impacts on single species (1,2). Studies carried out over three years in our laboratory with freshwater lake microcosms, ranging in size from 4 to 200 liters, have been directed mainly toward developing and evaluating microcosms as toxicological testing systems.

Microcosms can be a useful tool for impact evaluation only to the extent that: i) identically initiated systems replicate well, ii) their biological and chemical behavior, under both undisturbed and disturbed (with toxicant present) conditions, are similar to that of natural systems, iii) the attainment of i) and ii) does not result in excessive complexity or cost. The degree to which the inherent drawbacks of microcosms reduce their usefulness, in the above sense, will depend on the manner in which microcosms are set up and used. The objective of the research reported here has been to investigate some of the major options for freshwater lake microcosm design and operation, emphasizing the ways in which the choices among these options influence the usefulness of the system.

The criteria i-iii listed above will be referred to as replicability, realism, and simplicity, respectively. Simplicity is attained in our experimental design by the choice of microcosm initiation strategy and the operating conditions employed. To prevent excessive surface growth of algae in the microcosms, a simple technique of transferring water periodically to clean

containers was used. In most of the experiments reported, initiation of the microcosms was carried out by taking aliquots from a natural body--a procedure considerably simpler than the use of cultured organisms in gnotobiotic systems. We did not maintain a thermocline, hydraulic flow-through, benthic sediments, macrofauna, or fully realistic external temperature and light conditions in our microcosms. The appropriateness of all but the last two conditions (temperature and light) were discussed elsewhere (2) and will be summarized in the methods section. The effects of unnatural temperature and light conditions in the microcosms will be discussed in the results section.

The degree of realism of microcosm behavior is the degree to which the values of appropriate parameters measured in the microcosms track, or approximate, those measured in the natural lake which was the source of the microcosm contents. We refer to experiments designed to assess the realism of microcosm behavior as "tracking" experiments. To demonstrate the utility of microcosms for assessment of ecological impacts, realistic microcosm behavior under both undisturbed and perturbed conditions should be verified. The tracking experiments reported here are confined to investigation of undisturbed microcosms and lakes.

The pivotal question in microcosm research is: can results found in a microcosm be extended to a natural system? If good tracking is demonstrated for unperturbed microcosms, then reliable control systems would be established for microcosm toxicology studies. Moreover, the better a microcosm tracks a specific natural system, the more likely it is that effects of a toxin found in that microcosm will resemble those that the toxin would induce in the natural system (at least over a limited period of time). These toxic effects can then be extended to other similar natural systems, to the same degree that ecological phenomena in nature are transferable from one system to another. On the other hand, generic microcosms (those that do not track specific lakes but only resemble in a general way a class of lakes) are less likely to be of use in ecotoxicology. A simplified version of results from our experiments will elucidate that point, as well as motivate our choice of variables used to define tracking.

Consider a lake that for a period of 4 months has as its dominant phytoplankton a diatom population of constant biomass and constant (averaged over 24 hours)

primary productivity rate. Further, assume a microcosm derived from that lake tracks variables such as the major nutrient concentrations but does not track phytoplankton species. In particular, its phytoplankton quickly succeed to filamentous greens with the same biomass and daily average primary productivity rate as the lake's diatom population. Now consider adding a substance to this microcosm which happens to be toxic to filamentous greens but not diatoms. From this experiment, one would conclude that the lake's phytoplankton and primary productivity would be eliminated when in fact they would not. We belabor this point because of the frequently expressed belief that so-called "system" properties such as primary productivity are adequate to compare and extend results from microcosms to natural systems (3).

The selection of appropriate parameters to measure in tracking experiments and the choice of suitable statistical measures to express the degree of tracking are non-trivial. From the full range of measurements that we perform we have selected a subset of chemical and taxonomic parameters in terms of which we express the realism of our microcosms. These include concentrations of ammonia and nitrite plus nitrate, and the volumes of the major species of phytoplankton and zooplankton. Motivation for this choice of parameters is given below and elsewhere (2). These parameters are considered by us to be a minimal set for demonstration of tracking; particular attention must be paid to the succession patterns of the dominant taxa. As discussed in the results section, the major tracking problems for lake microcosms designed to be models of pelagic communities are manifest as distortions in plankton succession patterns. Significantly, the nature of these distortions points the way toward improvement of aquatic microcosm design and operation so as to enhance microcosm realism.

Two related tasks important to successful running of lake microcosms deserve special mention. These are eliminating size-dependent behavior and eliminating surface growth. Of the potential drawbacks to microcosm use, the growth of organisms on the inner surfaces of the microcosm containers is perhaps the most worrisome. The surface-to-volume ratio of small laboratory containers is large compared with natural lakes, and therefore excessive surface growth can exert an unnatural influence on the pelagic communities in the laboratory systems. Indeed, previous studies demonstrated that this can become a severe problem within several weeks after initiation of a microcosm (4).

Surface-growing algae are a nutrient sink during their rapid growth phase; on the other hand, attached microbial communities can be a major source of nutrients to the water column. Thus, excess surface growth can vitiate attempts to perform meaningful nutrient budget analyses in the pelagic community. Moreover, toxic substance impact evaluation is likely to be rendered difficult if unnaturally large, attached, algal populations function as a sink for such substances. Surface growth can lead to water column behavior in a microcosm that is strongly dependent on the size and shape of the microcosm container, thus shedding doubt on the validity or realism of results, particularly in experiments of greater than several weeks duration. We have discussed elsewhere our earlier unsuccessful attempts to eliminate surface growth (2). A major result of the present series of experiments is that the periodic transfer of the microcosm contents to clean containers is a successful method for eliminating surface growth. By this procedure, surface growth never builds up. Because the growth is exponential in its early stages, the total amount of biomass "thrown away" by this procedure can be made quite small simply by carrying out the transfer procedure at sufficiently frequent intervals. Although lake microcosms are not realistic when surface growth is not eliminated, we did carry out experiments with surface growth not eliminated in order to help us evaluate other researchers' work.

DESCRIPTION OF EXPERIMENTS

Tables 1-1 and 1-2 show the microcosm sizes and configurations used. Experiments I-V were aimed at developing and evaluating microcosms as assessment tools. Experiment VI was an exploratory application of microcosms; it is hoped that experiment VI, will lead to an improved way to test effects of toxins on nutrient regeneration rates in aquatic systems.

Experiments I-IV

These four experiments form the major portion of our work. As a group they were used to study: 1) replication, 2) effects due to elimination of surface growth, 3) size effects, 4) tracking, 5) generic succession patterns induced by laboratory conditions, and 6) effects due to water agitation.

Experiment V

This experiment involved a two-year run in which the possible persistence of cycles and long term effects of the pouring strategy for surface growth control

Table 1-1

SYNOPSIS OF EXPERIMENTAL CONDITIONS FOR EXPERIMENTS I - V

Experiment	Duration	Inclusive Experimental Dates	Container Sizes(L)	Series Designation	Number Replicates	Surface Growth Mitigation	Aeration	Tracking Lake	Parameter Sampling Interval	Container Material	Source of Water
I	13 weeks	26/V/78	200	A	3	Control	+	None	Weekly	polyethylene	mixture of various local lakes
		26/V/78	200	B	3	Pour	+				
		9/VIII/78	15	C	3	Decant	+				
			15	D	3	Pour	+				
			15	E	3	Control	+				
II	13 weeks	19/X/78	50	A	3	Control	+	Lafayette Reservoir	weekly	polyethylene (A,B,C)	Lafayette Reservoir
		19/X/78	50	B	3	Pour	+				
		17/I/79	50	C	3	Control	-				
			4	D	3	Control	+			glass (D,E,F)	
			4	E	3	Pour	+				
			4	F	3	Control	-				
III	11 weeks	20/IV/79	15	A	3	Pour	+	Briones Reservoir	weekly	polyethylene	Briones Reservoir
		20/IV/79	50	B	3	Pour	+				
		20/IV/79	50	C	3	Pour (Inoculated)	+				
		5/VII/79	150	D	3	Pour	+				
IV	8 weeks	14/XI/79	50	A	3	Control	-	Briones Reservoir	weekly	polyethylene	Briones Reservoir
		11/I/80	50	B	3	Siphon	-				
			50	C	3	Pour	-				
			50	D	3	Pour 2	-				
V	~2 years	25/V/78	200	A	1	Control	+	none	variable	polyethylene	mixture of various local lakes
		14/V/80	200	B	1	Pour	+				
		5/X/78	15	C	2	Control	+				
		14/V/80	15	D	2	Pour	+				
VI	(See Appendix A)										
VII	(See Appendix B)										

Table 1-2

SUMMARY OF THE CONFIGURATIONS OF THE 4 DETRITUS-ADDITION SUB-EXPERIMENTS

Experiment	Initial organic carbon concentration	Detrital material	system	Amount of detritus added, expressed as increase in organic carbon		replicates
K-1	430 μM(C)	E. coli (DOM+POM)*	A	0	(control)	2
			B	27		2
			C	54		2
			D	109		2
K-2	340 μM(C)	E. coli (DOM+POM)*	A	0	(control)	3
			B	24		3
			C	48		3
			D	108		3
			E	180		3
			F	300		3
K-3	260 μM(C)	algae (DOM)*	A	0	(control)	2
			B	58		2
			C	116		2
			D	348		2
K-4	260 μM(C)	algae (DOM+POM)*	A	0	(control)	2
			B	61		2
			C	122		2
			D	366		2

*DOM = dissolved organic matter (see Section 5).
POM = particulate organic matter (see Section 5).

were investigated. Earlier studies in our laboratory (4) had demonstrated the persistence of population cycles over a 6-month period; experiment V was designed in large part to extend that study in order to ascertain the validity of the widely held view (5,6) that microcosms tend to evolve toward static populations and nutrient levels.

Experiment VI

Experiment VI explored the use of microcosms for the study of decomposition processes in the pelagic communities of lakes (7). Whereas the experimental runs in I through V each lasted two months or longer, the decomposition experiments were of short duration, generally lasting two to three weeks. The results not only were of interest in their own right (see Section 6), but also point the way toward a possible application of microcosms to the problem of developing a standard screening procedure for characterizing the effects of pollutants on nutrient cycling rates.

ROLE OF MICROCOSMS IN IMPACT ASSESSMENT

Many important questions concerning ecological effects of pollutants can never be answered in microcosms alone, because of their inherent limitations. For lake microcosms, these limitations include their increasing unreality over time (and thus their ineffectiveness for studies of long-term effects) and their relatively small size, which precludes inclusion in a realistic fashion of fish and large aquatic plants, except at enormous cost and difficulty. At first glance, it might appear that microcosms would be of little use in assessing many of the impacts of greatest concern to the public. General public concern over possible damage to lakes centers on a relatively small number of issues, including direct chemical threats to drinking water quality, impairment of sports-fish populations, aesthetic loss from increasing turbidity or eutrophication of the water, enhanced odor-producing biological activity, and increased likelihood of disease-bearing vectors and pathogens in the lake.

However, these threats can be anticipated adequately only to the extent that ecosystem processes in the lake are understood, for the degree to which these problems will materialize depends on a multitude of linkages among the biotic and abiotic components of the lake ecosystem. Even the first of these issues, drinking water quality, can involve ecological linkages. For example, estimates of the equilibrium water concentration of toxic metals based simply

on the input rate and the hydraulic residence time of the lake could be misleading because increased acidity of lake waters can cause the leaching of toxic metals from lake sediments into solution where they are then accessible to the public. The reduction of sport fish can take place by a variety of mechanisms in addition to direct toxic effects on fish growth or reproduction. For example, a toxicant that diminishes the rate at which microbes generate ammonia from lake-water detritus might ultimately reduce the growth rate of sport fish. The reason is that ammonia is a prime source of nitrogen for phytoplankton which, in turn, is the food source for zooplankton, and thus the food chain leading to fish might be impaired by damage at the microbial level.

These examples illustrate the point that much of the information needed to assess environment impacts of concern to the public can be generated from ecological studies involving realistic segments of natural systems such as the microbial and detritus components, or the sediment-water interface, or the phytoplankton and zooplankton in the water column of the lake. Microcosms can play a major role in this regard.

The most effective use of microcosms in impact assessment will be achieved with sensible integration of field work, single-species testing, and laboratory microcosm analyses. Suppose, for instance, one wants to determine the likely damage to a sport fish population from a toxicant. Microcosms can be of great use in determining the effect of the substance on nutrient regeneration and also the direct effects on the plankton. Information derived from field studies, then, can provide the linkages between the fish, on the one hand, and the plankton growth rates and the nutrient chemistry of the lake, on the other hand. Such information can be the result of many years of field experience with a variety of lakes and need not be obtained in the context of the particular pollutant, whereas the pollution studies could be carried out entirely in the laboratory.

Another example of sensible integration of test procedures involves single-species tests. Results of single-species tests are important to allow "targeting" on sensitive organisms, thus avoiding unnecessary monitoring of all species in microcosm studies. The microcosm, in this sense, can provide the realistic environment for the targeted species as well as a realistic aqueous environment in which chemical transformations of the pollutant can take place.

FUTURE EFFORT

The method of microcosm initiation that we employ results in microcosms that behave like the natural lake from which the microcosm materials were obtained for a period of up to two months under appropriate conditions. The degree of realism and replicability that is achievable is sufficient to allow a wide range of applications of lake microcosms to environmental impact assessment. This range would be extended if lake microcosms that incorporated lake-bottom substrate could be shown to be as realistic and replicable as the water-column systems we have studied here. Microcosms with substrate will not be as simple to construct or operate as ones without substrate, if the interaction of the substrate with the overlying water is to resemble that of natural lakes. The development of suitable lake-substrate systems and the determination of their realism and replicability will be the subject of future research by our group. The importance of the lake-bottom as a sink for nutrients and as an important site for nutrient regeneration underscores the need to extend in this direction the range of applicability of microcosms.

Although benthic microcosms for freshwater lakes are still in the development, or preoptimization, stage, pelagic systems are now sufficiently well understood that the actual structuring of test protocols for testing certain kinds of pelagic effects can begin. Inorganic nutrient regeneration, an important ecosystem-level function, can be degraded by toxic substances. We are beginning a research effort to develop and evaluate a test procedure for determining the effects of pollutants on the nutrient-regeneration capability of lakes. Our effort will focus on nutrient regeneration in the water column of lakes, since that is the lake compartment for which microcosm models are best understood. Much information can be learned from short-term tests (of not more than one-month duration) of nutrient-regeneration impairment. Because the realism and replicability of microcosm behavior over much longer time periods is yet to be achieved, and may be impossible, testing effects on this particular ecosystem property is particularly well-suited to microcosms.

An equally important task for the future is the demonstration of the tracking of a polluted lake by similarly polluted microcosms. It is our judgment that such work should await further understanding of the behavior and realism of unperturbed microcosms and the development of suitable test protocols based on optimized test systems. In that way, the more difficult and possibly

environmentally harmful tests carried out under perturbed circumstances will involve microcosms that have been adequately studied and optimized so that a basis for confidence in them will exist.

Section 2

MICROCOSM ASSESSMENT STUDIES: EXPERIMENTAL CONDITIONS AND METHODS

EXPERIMENTAL CONDITIONS

The procedures described below were used in experiments I-V. All systems were maintained in a temperature-controlled room, with temperature range from 18°-20°C. Illumination was provided by banks of 1.3 m, very high output, cool white, fluorescent lights on a 12h:12h light:dark cycle; the light irradiance on the water surface of the microcosms was 6 ± 1 watts/m^2 PAR. This was approximately one-thirtieth of the estimated annually-averaged natural levels at the lake surface. Aeration, where used, was provided by gently passing air through a capillary tube which extended below the water surface. The flow rate was about 1 liter/minute. Containers varied in size and construction material (Table 1-1). Cylindrical polyethylene containers were used in all experiments except VI and VII, the containers varied in size from 4 to 200 liters. Four-liter glass beakers and flasks were used in VI and VII. Systems were run both with and without surface-growth mitigation. Three strategies for eliminating surface growth were studied. One, which we refer to as "pouring", consists of simply dumping the entire flowing contents of the tanks into clean containers. In the second, "decanting", the contents of the tanks were decanted into clean containers, avoiding the transfer of settled detritus. In the third, "siphoning," the container contents were siphoned into clean containers, avoiding violent agitation of the water. As a fourth treatment, in one experiment (IV) the contents of the containers were poured into clean tanks and then immediately poured back into the original containers, thus duplicating the agitating effect of pouring without eliminating surface growth. Deionized water was used to make-up losses from evaporation and sampling.

We do not maintain a thermocline, hydraulic flow-through, macrofauna, or lake sediments in our systems. Our rational for these exclusions and other comments on our methods follow below.

Thermocline Even though the creation of a thermocline is possible, the presence of a well-illuminated hypolimnion of small volume would be so unrepresentative of most natural systems that the additional effort is unwarranted. This is particularly true in the cases where periodic pouring is used for surface-growth prevention and stratification would be destroyed at frequent intervals.

Hydraulic Flow-through. Hydraulic flow-through appears unwarranted in our systems for the following reason. Typical residence times for the water of natural lakes are on the order of one year or more. Indeed, the biological activity during summer stratification of most temperate lakes appears to be determined by the concentrations of dissolved nutrients already present at the onset of spring overturn (8). Although inflowing water constantly transports plant nutrients to the photic zone of lakes, the percentage addition rate is so slow in most cases that the major effect is to influence the long-term average fertility of the water, rather than to affect the temporary character of the water quality or biota within a given season. Important exceptions do occur, however, and in our experiment-II, nutrient influx from watershed runoff impaired the tracking of the microcosms.

Benthic sediments. The depth of water in most natural lakes is much greater than that found in microcosms. In addition, during summer stratifications benthic activity is isolated from most of the photic zone. Bottom sediments from natural lakes contain levels of organic matter and inorganic nutrients partially determined by the productivity and depth of the overlying water. When this material is removed and placed in a laboratory microcosm with a smaller mean water depth, the danger exists that the sediment will exert a larger effect on this water, surpassing its original effect on the water column of the parent system. Perez et al. (9), using 150-liter marine microcosms, have demonstrated that the presence of benthic sediments significantly affects the behavior of their microcosms. Since, in the tracking experiments reported here we are trying to simulate the upper levels of the water column over short periods of time, it is more realistic to eliminate natural benthic sediments from our shallow microcosms than to include them (4).

Water Agitation and Aeration. In some tanks, air is bubbled through capillary tubes at a rate of 1 liter/min. The transferring of water from one tank to a

clean one in order to eliminate surface growth also introduces agitation. As we will discuss below and as noted by other groups (9), the amount of agitation can affect microcosm behavior.

Macrofauna We exclude macrofauna from our systems. A detailed discussion of the merits of this procedure has been discussed elsewhere (2). In summary, inclusion of macrofauna (e.g., fish, gastropods) in microcosms less than 10 m^3 volume reduces rather than increases resemblances to natural systems.

Light Levels In addition to spectral and temporal distortions introduced by the use of artificial illumination, the vertical light profile is unrealistic in our shallow microcosms. This feature is inherent to lake microcosms because transmission through, and reflection by, the sides of the containers is significant and because transmission phenomena are spectrally dependent. Systematic determination of the most appropriate illumination for lake microcosms has not been carried out to our knowledge. Our work and work with marine microcosms suggests that using lower intensities than those found naturally may be reasonable. For example, Perez et al. (9), report that significantly less than (~ 1/7) natural levels of illumination in their microcosms produce phytoplankton behavior which is more commensurate with their natural parent system than that produced by more natural levels of illumination.

MEASUREMENTS

The following parameters were measured in all our experiments: NH_3, NO_3^- + NO_2^-, temperature, and phytoplankton and zooplankton species and numbers. In some experiments the following additional parameters were measured: pH, inorganic carbon, organic carbon, fluorescence, O_2, and total phosphorous. The methods used are given in Table 2-1. In Experiments I-IV all parameters were measured weekly at the same time that the parent lake samples and readings were taken. In experiment V, fluorescence was measured twice weekly, while plytoplankton and zooplankton taxa and numbers were measured bi-weekly.

FIELD SAMPLING

Two near-by oligotrophic lakes within 30 km of the laboratory were used in the tracking experiments (II-IV): Lafayette Reservoir, 5 km in circumference, 36 m maximum depth, containing 4.2 x 10^6 m^3 of water, and Briones Reservoir,

Table 2-1

EXPERIMENTAL METHODS

Parameter	Method	Special Equipment	Reference
O_2	polarography	O_2 meter (YSL 57)	--
H	electrometry	pH meter (Orion)	--
IC	infrared absorbance	IR analyzer (Beckman 865)	--
OC	combustion to IC	TOC analyzer (Beckman 915A)	--
NH_4^+	blue indophenol reaction	spectrophotometer (Zeiss PM2 DL)	(10)
$NO_3^- + NO_2^-$	reduction, diazotization	"	(11)
CO_2 evolution	equilibria kinetics	pH meter (Orion 601) IR analyzer (Beckman 865)	(12)
phytoplankton	tube chamber	5 ml tube chamber (Wilde) inverted microscope (Lietz)	--
zooplankton	counting chamber	100 ml count. chamber (Wild) binocular microscope (Lietz)	--

22 km in circumference, 70 m maximum depth, containing 8.4×10^7 m^3 of water. The annual input to Briones Reservoir in the form of runoff from its watershed and staged releases from Pardee Reservoir amounts to about 5% of the volume of Briones. In an average year, 60% of that input is in the form of runoff and 40% is from Pardee. The total annual input to Lafayette reservoir is about 30% of its volume, with 85% coming from local runoff and 15% from Pardee.

Water from the epilimnion of Lafayette Reservoir was used to initiate Experiment II, while water from the epilimnion of Briones Reservoir was used to initiate experiments III and IV. Water was retrieved from the field sites in large containers and distributed immediately into the microcosm tanks in the laboratory. To monitor lake parameters, field samples were taken at weekly intervals during times of our laboratory tracking runs. Lake samples were taken on the same day as laboratory microcosm samples. Irregular field sampling was done between experiments to ensure a continuous record of lake activity. All field samples were taken from a small boat at a preselected site a sufficient distance from shore (~100 m) to insure that samples were in the pelagic zone of the lake. Depth of the water at the two reservoirs was greater than 20 m. Samples were taken by lowering a 2-liter Van Dorn sampler over the side of the boat to a depth of 1 m. Two samples were taken, one for zooplankton, a second for phytoplankton and water chemistry. Temperature was taken with a thermometer as soon as water samples were taken. The 2-liter zooplankton sample was filtered through a 64μ mesh net and placed in a 1-liter bottle to be returned to the laboratory for counting.

LABORATORY SAMPLING

Depth-integrated laboratory samples for chemical analyses for all experiments were taken by inserting a hollow polyethylene tube into the water column of the microcosm. Modifications were made for sampling for experiment VII, in which Ehrlenmayer flasks were used, by inserting a large, pre-cleaned glass cylinder into the microcosm. Phytoplankton samples were taken in a manner similar to chemical samples. Photoplankton samples were examined in 5 ml Utermoehl chambers first at low power to count all large species, and then part of the chamber was examined at high power (one or two sweeps down the diameter of the chamber) in order to obtain counts of smaller species. Identifications were usually made only to genera using standard reference texts (13,14) and where

difficulties were encountered laboratory co-workers, photographs, and occasionally outside experts were used to make or confirm identifications. Random measurements of 10 cells of each phytoplankton species were taken, and cell volumes were calculated to 2 significant figures by matching an appropriate geometric formula to the cell shape.

Initial zooplankton samples in the earlier experiments (I-III) were taken with a premeasured 100 ml glass cylinder from the surface of the microcosm. After initial data analysis it was decided that a larger zooplankton sample was needed. Accordingly, zooplankton samples were taken in experiment IV with a vertical tow of 5 cm diameter a plankton bucket (Wildco) fitted with a 64μ Nitex straining net. These samples were then filtered through a 64μ net down to 100 ml and placed in 100 ml settling chambers for counting. All phytoplankton and zooplankton samples sat 24 hours before counting. Zooplankters smaller than 64μ in greatest dimension, such as some protozoa, were counted along with the phytoplankton. Identification procedures proceeded as outlined for phytoplankton using standard identification manuals (14,15). Field phytoplankton and zooplankton samples were counted along with laboratory samples. All phytoplankton and zooplankton samples settled 24 hours before counting.

DATA ANALYSIS

In order to evaluate microcosm performance, we need to compare putative replicate microcosms with each other and with the natural system from which they were derived. The putative replicate microcosms were initiated and maintained under as similar circumstances as possible. In traditional statistical analyses, the differences between such replicate systems are, in effect, attributed to measurement error only. Because of the large number of components in our systems and the complexity of their interactions, measured differences among such putative replicate systems can occur, which are not due to measurement uncertainty but which reflect fundamental behavioral differences among the putative replicates. Thus for systems such as ours, traditional statistical techniques would be misleading, as they often lump measurement uncertainties with fundamental differences in behavior of the replicate systems.

In appendix A, we describe our data analysis procedure, which allows us to scale the measured differences between replicates by the precision in our

measurement techniques. The development of this procedure, required that we carefully calibrate our measurement methods. We determined that the measurement uncertainties associated with three important variables NH_4^+, $NO_3^-+NO_2^-$, and phytoplankton volume densities depended on the mean value of the variable measured. In contrast, many traditional analyses assume such uncertainties are independent of the mean value of the measured variable.

In the appendix, we present explicit criteria for rating the goodness of replication among putative replicates as well as a system for rating the degree of tracking between the replicate system and the parent natural system. Both sets of criteria are quite stringent. They include both uncertainties due to measurement and differences due to behavior variations among replicates. In particular for good tracking, we require that not only the mean value among replicates be near the natural system's parameter value but that the variation among replicates be small.

Section 3

MICROCOSM ASSESSMENT STUDIES: RESULTS

EXPERIMENTS I-IV

We group our discussion of nutrient and photoplankton measurements from experiments I-IV, into four categories 1) replication, 2) size differences, 3) tracking of parent water body, 4) effects due to agitation. Zooplankton data are discussed separately due to problems unique to their measurement.

For much of this discussion, we use data which are averaged over short time intervals (Appendix A). There are two reasons to average data over time. First the jitter inherent in natural systems' parameters is smoothed out to some degree by this process. Secondly, it allows for sensible comparison between different systems which may differ from one another in one or more of their variables only by a phase difference in time. For example, two freshwater systems may both exhibit a similar diatom bloom but be slightly out of phase if the bloom in one precedes that in the other by a few days. The time-aggregated value for diatom volumes would be similar for both systems, whereas a day-by-day comparison of the diatom populations would yield different results. For our microcosms and lakes such phase differences were judged not to be biologically significant, and so we used time-aggregated quantities in our evaluation of replication and tracking.

In Table 3-1, the time intervals over which data were aggregated are given for each of the four experiments. The choice of each time interval is somewhat arbitrary. In general, they were selected such that major phytoplankton blooms are included within the interval. The endpoints of the intervals usually occurred at times when the systems were quiescent. The time aggregated values for the nutrients and phytoplankton volumes are given in Tables 3-2 through 3-25. Phytoplankton volume densities are given for each dominant species, as well as for those species deemed interesting for other reasons, within the time interval of interest. Appendix B contains all the data from experiments I-IV.

Table 3-1

TIME INTERVALS

Experiment	Interval (days)
I	1 - 21, 21 - 56, 56 - 63
II	7 - 22, 22- 56, 56 - 92
III	7 - 14, 14 - 56, 56 - 77
IV	7 - 21, 21 - 59

Tables 3-2 through 3-25 contain the following quantities: 1) The time-aggregated parameter value for the i'th replicate (x_i), 2) the standard deviation (σ_i) due to measurement uncertainties associated with that parameter, 3) when appropriate the mean value for the three replicates ($\bar{x}$), 4) the square root of the variance among replicates (S), and 5) the replication number $R = S/\bar{\sigma}$ (Appendix A). In some cases, a replication rating (letter in single parentheses) and/or tracking rating (letter in double parentheses) is included in these tables (Appendix A). In experiments II and III, phytoplankton samples from the smallest tanks (4 liters and 15 liters, respectively) were pooled together for each set of three replicates. This was because we only wanted to remove small volumes of water from each tank.

Replication

Our criteria for biologically significant replication are described in Appendix A. Here we describe our results with respect to these criteria.

For each experiment, for each time interval during that experiment, for each variable of interest, and for each treatment set within the experiment. we assign a replication rating running from excellent to poor. Having assigned such ratings, as is done where appropriate in Table 3-2 through 3-25, we can summarize the information as shown in Tables 3-26 and 3-27. In these two tables, the number of ratings in each category are added up over all the

Table 3-2

EXPERIMENT I, NH_4^+(μM(N))

	Days 1-21					Days 21-56					Days 56-63				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	2.42	.162				3.21	.14				2.75	.28			
2	3.65	.188	3.0	.06	3.42	3.15	.14	3.0	.3	2.19	5.65	.333	4.3	1.5	4.93
3	2.77	.175			(G)	2.69	.13			(G)	4.50	.298			(F)
B_1	2.95	.175				3.50	.188				2.45	.228			
2	2.97	.175	2.7	.5	2.99	3.77	.20	3.6	.2	1.04	2.90	.245	2.8	.3	1.25
3	2.07	.150			(G)	3.37	.188			(E)	2.95	.245			(G)
C_1	2.67	.162				2.71	.13				3.35	.262			
2	2.07	.150	2.2	.4	2.60	3.10	.14	2.9	.2	1.46	2.10	.210	2.8	.6	2.50
3	1.85	.150			(G)	2.83	.14			(E)	3.00	.245			(G)
D_1	3.10	.175				3.19	.14				6.07	.35			
2	2.78	.175	2.7	.5	2.99	2.73	.13	3.2	.5	3.23	3.55	.262	4.04	1.8	6.32
3	2.17	.150			(G)	3.72	.188			(G)	2.50	.228			(F)
E_1	2.05	.150				2.54	.13				3.40	.262			
2	3.28	.188	2.7	.6	3.50	4.74	.17	3.4	1.2	8.13	10.70	.455	5.8	4.3	12.86
3	2.78	.175			(G)	2.83	.14			(F)	3.15	.245			(P)

Table 3-3

EXPERIMENT I, $NO_3^- + NO_2^-$(μM(N))

	Days 1-21					Days 21-56					Days 56-63				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	2.65	.375				2.81	.294				2.75	.49			
2	8.45	.915	5.0	3.0	4.61	3.13	.294	3.0	.2	.680	13.30	1.33	7.1	5.5	5.86
3	4.00	.54			(P)	3.19	.294			(E)	5.25	.735			(P)
B_1	4.20	.54				2.12	.231				3.00	.49			
2	4.30	.585	4.2	.2	0.36	3.13	.294	2.8	.6	2.18	5.95	.84	4.7	1.5	2.13
3	4.00	.54			(E)	3.19	.294			(G)	5.25	.735			(F)
C_1	4.65	.585				3.01	.294				3.35	.56			
2	3.95	.54	4.2	.4	0.72	3.85	.378	3.5	.4	1.18	4.40	.682	5.3	2.5	3.22
3	4.00	.54			(E)	3.57	.336			(E)	8.15	1.02			(F)
D_1	3.80	.54				2.66	.273				9.40	1.16			
2	5.10	.63	4.3	.7	1.22	3.02	.294	3.0	.3	1.02	13.95	1.33	10.6	2.94	2.48
3	4.00	.54			(E)	3.21	.315			(E)	8.45	1.05			(P)
E_1	3.80	.51				3.13	.302				5.85	.805			
2	3.80	.51	4.0	.4	.75	2.77	.290	2.9	.2	.68	2.75	.455	4.1	1.6	2.56
3	4.45	.57			(E)	2.91	.294			(E)	3.55	.56			(F)

Table 3-4

EXPERIMENT I, SMALL BLUE GREENS ($\times 10^6$ μ^3/ml)

	Days 1-21					Days 21-56	
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i
A_1	.9	.05					
2	.75	.04	.79	.10	2.29	.03	.02
3	.72	.04			(G)	.04	.02
B_1	1.14	.06				.07	.02
2	1.15	.06	1.01	.24	4.43	04	.02
3	.73	.04			(G)	.04	.02
C_1	.19	.01				-	
2	2.24	.12	1.10	1.05	13.95	.06	.02
3	.86	.05			(P)	-	
D_1	.62	.03			-		
2	.22	.01	.32	.27	14.10	-	
3	.11	.01			(P)		
E_1	.29	.02				-	
2	.51	.03	.51	.22	7.08	-	
3	.72	.04			(A)		

Table 3-5

EXPERIMENT I, ANKISTRODESMUS (x 10^5 μ^3/ml)

	Days 1-21					Days 21-56	
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i
A_1	~.1	.1					
2	.1	~.1					
3	0	.1					
B_1	.17	.02					
2	.14	.02	.16	.017	.85		
3	.17	.02			(E)		
C_1	15	2.1				3.92	.39
2	1.18	.12	6.63	7.36	5.98	.6	.08
3	3.7	.31			(P)	.9	.13
D_1	4.2	.42					
2			2.51	2.22	7.53		
3	3.33	.29			(P)	1.16	.12
E_1	3.43	.34				2.81	.21
2	2.43	.34	2.54	.85	.85		
3	1.77	.25			(E)	.32	.04

Table 3-6

EXPERIMENT I

	MOUGEOTIA ($\times 10^6$ μ^3/ml)					SYNEDRA RADIANS ($\times 10^5$ μ^3/ml)				
	Days 1-21					Days 21-56				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	-					.62	.04			
2	.58	.15	.39	.34	2.78	.14	.02	.41	.236	6.81
3	.6	.15			(P)	.46	.04			(A)
B_1	2.6	.36				-				
2	3.94	.39	3.3	.67	1.86	-				
3	.36	.09			(A)					
C_1	4.24	.37				-				
2	6.26	.46	3.62	3.00	8.70	-		.057	.098	5.66
3	.36	.09			(P)	.17	.03			
D_1	4.8	.48				-				
2	2.6	.26	3.23	1.37	4.08	-		.127	.22	7.62
3	2.3	.20			(A)	.38	.05			
E_1	.65	.16				1.25	.09			
2	.61	.16	.42	.36	2.79	.38	.03	.82	.386	5.96
3					(P)	.78	.06			(A)

Table 3-7

EXPERIMENT II, NH_4^+(μM(N))

	Days 7-22					Days 22-56					Days 56-92				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	4.20	.246				2.23	.124				2.77	.136			
2	5.80	.294	4.7	.9	3.42	2.42	.128	2.3	.1	.80	2.68	.136	2.9	.2	1.44
3	4.23	.246			(A)	2.28	.124			(E)	3.09	.144			(E)
B_1	7.65	.348				3.04	.14				4.60	.172			
2	6.85	.318	6.8	.9	2.82	2.91	.136	4.1	2.0	12.1	5.92	.20	4.5	2.1	12.2
3	5.83	.288			(A)	6.45	.208			(F)	3.04	.14			(F)
C_1	3.43	.222				2.58	.132				2.70	.136			
2	3.95	.24	3.6	.3	1.30	2.35	.128	2.3	3	2.34	2.48	.128	2.6	.1	.76
3	3.50	.228			(E)	2.09	.124			(G)	2.58	.132			(G)
D_1	3.58	.228				2.30	.128				2.60	.132			
2	3.58	.228	3.5	.2	.89	1.98	.12	2.0	.2	1.66	3.09	.14	3.0	.4	2.85
3	3.23	.216			(E)	1.83	.112			(E)	3.30	.148			(G)
E_1	3.30	.222				2.58	.132				4.32	.168			
2	4.18	.246	3.7	.4	1.72	3.44	.148	3.0	.4	2.83	4.81	.176	4.7	.3	1.72
3	3.63	.228			(E)	3.11	.144			(G)	4.96	.18			(E)
F_1	4.03	.24				3.31	.148				3.15	.144			
2	3.15	.216			2.72	3.03	.14	3.2	.2	1.38	3.01	.14	3.4	.5	3.37
3	2.90	.204			(G)	3.37	.148			(E)	3.99	.16			(G)
Field	3.68	.23				7.9	.25				13.41	.32			

Table 3-8

EXPERIMENT II, NO_3^- + NO_2^-(μM(N))

	Days 7-22					Days 22-56					Days 56-92				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	3.23	.525				3.13	.432				4.93	.40			
2	4.05	.63	3.6	.2	.35	3.13	.432	3.1	.1	.23	4.68	.384	4.5	.5	1.32
3	3.48	.56			(E)	2.95	.414			(E)	3.89	.352			(E)
B_1	4.58	.672				4.60	.582				7.58	.56			
2	3.98	.63	4.2	.3	.47	3.95	.54	4.7	.75	1.25	6.33	.50	6.7	.7	1.34
3	4.03	.63			(E)	5.45	.672			(E)	6.30	.50			(E)
C_1	3.40	.546				3.30	.456				3.38	.312			
2	3.50	.56	3.2	.4	.75	3.20	.444	3.2	.1	.22	3.73	.34	3.7	.3	.89
3	2.80	.469			(E)	3.15	.438			(E)	3.99	.36			(E)
D_1	3.76	.595				4.70	.606				7.78	.572			
2	3.18	.511	3.5	.3	.53	5.58	.684	4.9	.6	.96	3.61	.328	5.3	2.2	4.99
3	3.68	.588			(E)	4.53	.588			(E)	4.40	.384			(F)
E_1	3.18	.518				8.08	.876				18.34	1.06			
2	3.30	.532	3.4	.2	.37	7.23	.822	8.1	.9	1.01	18.66	1.07	20.7	3.8	3.29
3	3.60	.574			(E)	9.03	.96			(E)	25.03	1.32			(A)
F_1	4.08	.637				4.45	.582				5.04	.424			
2	3.18	.518	3.5	.5	.89	4.05	.546	4.2	.2	.36	4.41	.384	4.6	.4	1.00
3	3.28	.532			(E)	4.18	.558			(E)	4.46	.388			(E)
Field	3.2	.5				4.23	.52				5.56	.46			

Table 3-9

EXPERIMENT II

($\times 10^5$ μ^3/ml)

	FRAGILARIA					FRAGILARIA STEPHANODISCUS		FRAGILARIA STEPHANODISCUS ASTERIONELLA	
	Days 7-22					Days 22-56		Days 56-92	
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	x_i	σ_i
A_1	.77	.07							
2	.26	.04	.43	.29	5.29				
3	.26	.05			(P)				
B_1	.80	.07							
2	.51	.05	.67	.15	2.48				
3	.70	.06			(P)				
C_1	.5	.07							
2	.4	.06	.40	.10	1.65				
3	.31	.05			(E)				
D	-								
E	.5	.05							
F	.09	.02							
Field	6.08	.37				17.46	.88	14.33	.76

Table 3-10

EXPERIMENT II,

	ANABAENA ($\times 10^5$ μ^3/ml) Days 22-56 None in tanks		CERATIUM ($\times 10^5$ μ^3/ml) Days 56-92 None in tanks				
	x_i	σ_i	x_i	σ_i			
Field	1.15	.05	1.7	.18			
			Coscinodiscus ($\times 10^5$ μ^3ml)				
			x_i	σ_i	$\bar{x}$	S	R
A_1			-				
2			-				
3			-				
B_1			-				
2			-				
3			-				
C_1			37.	9.78			
2			2.9	.78	14.23	19.72	3.47
3			2.8	.74			
D			3.6	.95			
E			-				
F			.24	.12			
Field			-				

Table 3-11

EXPERIMENT II,

	FILAMENTOUS (x 10^5 μ^3/ml)					FLAGELLATES (x 10^5 μ^3/ml)				
	Days 56-92					Days 56-92				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	~10^4	-				.38	.05			
2	12.29	.61	5.98	6.15	15.67	.24	.03	.33	.08	2.00
3	5.65	.30			(P)	.38	.05			(A)
B_1	.55	.08				1.24	.12			
2			.183	.318	6.88	.38	.05	1.05	.62	5.64
3	-				(P)	1.56	.16			(A)
C_1	-					4.63	.34			
2			.44	.762	10.15	2.14	.16	3.78	1.42	5.46
3	1.32	.13			(P)	4.56	.30			(A)
E	-					.55	.08			
F	.2	.03				.95	.08			

Table 3-12

EXPERIMENT III, NH_4^+(μM(N))

	Days 7-14					Days 14-56					Days 56-77				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	4.55	.301				3.65	.152				2.90	.175			
2	4.35	.294	4.2	.4	1.38	3.79	.156	3.7	.07	.46	2.60	.165	2.8	.16	.94
3	3.80	.273		((E))	((E))	3.69	.152		((E))		2.87	.170		((E))	(E)
B_1	4.65	.301				5.33	.188				5.20	.23			
2	5.15	.322	5.1	.4	1.25	5.51	.192	5.2	.4	2.17	2.93	.175	3.5	1.56	8.24
3	5.50	.336		((E))	(E)	4.68	.172		((E))	(G)	2.23	.156		((E))	(F)
C_1	4.80	.308				3.53	.152				3.40	.185			
2	5.50	.329	5.4	.5	1.53	4.43	.168	4.1	.5	3.07	6.03	.25	5.4	1.77	7.44
3	5.75	.343		((E))	(E)	4.34	.168		((E))	(G)	6.77	.27		((A))	(F)
D_1	6.5	.371				4.09	.16				2.30	.16			
2	6.35	.364	6.0	.7	1.98	3.68	.152	5.2	2.2	11.9	3.57	.19	4.5	2.7	12.2
3	5.15	.322		((E))	(E)	7.68	.232		((A))	(F)	7.55	.29		((A))	(P)
Field	5.15	.32				3.29	.15				2.95	.15			

Table 3-13

EXPERIMENT III, $NO_3^- - NO_2^-$ (μM(N))

	Days 7-14					Days 14-56					Days 56-77				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	4.55	.588				6.29	.496				13.82	1.08			
2	3.90	.528	4.1	.4	.73	7.08	.544	6.7	.4	.77	9.73	.835	12.4	2.3	2.29
3	3.80	.516		((A))	(E)	6.62	.516		((P))	(E)	13.7	1.08		((P))	(A)
B_1	3.55	.486				4.63	.396				8.70	.78			
2	2.70	.396	3.4	.7	1.47	3.91	.352	4.	.6	1.70	6.45	.635	5.9	3.1	5.09
3	4.00	.54		((G))	(E)	3.37	.308		((A))	(E)	2.53	.315		((P))	(P)
C_1	4.36	.57				3.25	.30				3.90	.44			
2	4.70	.606	4.1	.8	1.47	3.39	.312	3.3	.1	.33	6.35	.625	5.6	1.5	2.61
3	3.20	.444		((A))	(E)	3.16	.292		((E))	(E)	6.53	.64		((P))	(F)
D_1	3.85	.522				5.32	.44				4.52	49			
2	3.35	.462	3.3	.6	1.30	3.55	.324	4.2	1.	2.67	3.12	.36	4.8	1.8	3.49
3	2.75	.396		((E))	(E)	3.86	.348		((A))	(F)	6.73	.655		((P))	(F)
Field	1.65	.32				1.44	.18				2.25	.29			

Table 3-14

EXPERIMENT III, STEPHANODISCUS (x 10^5 μ^3/ml)

Days	7-14					14-56				56-77	
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A	12.	0.9		((E))		1.6	.08		((G))	
B_1	15.	1.1				2.5	.13			
2	11.5	1.0	14.	2.2	2.06	1.59	.08	2.08	.47	4.59
3	15.5	1.1		((E))	(G)	1.83	.09		((G))	(G)
C_1	16.1	1.1				2.62	.13			
2	11.9	0.8	15.	2.7	2.58	2.52	.13	2.74	.30	2.19
3	17.0	1.2		((E))	(G)	3.08	.15		((G))	(G)
D_1	17.1	1.2				2.08	.1			
2	12.	0.9	15.	2.8	2.61	1.08	.11	1.82	.65	13.95
3	15.5	1.		((E))	(G)	2.3	.12		((A))	(A)
Field	15.1	1.1				3.6	.14			

Table 3-15

EXPERIMENT III FLAGELLATES (x 10^5 μ^3/ml)

Days	7-14					14-56				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A	.37	.03		((E))		.32	.02		((G))	
B_1	-					.27	.02			
2	-			((P))		.10	.02	.157	.098	4.91
3	-					.10	.02		((P))	(P)
C_1	.67	.05				1.27	.06			
2	.68	.05	.67	.015	.31	1.27	.06	1.27	0	(0)
3	.65	.05		((F))	(E)	1.27	.06		((F))	(E)
D_1	.61	.04				1.12	.05			
2	-		.20	.352	15.42	.42	.03	.51	.57	9.78
3	-			((P))	(P)			((P))	(P)	
Field	.39	.03				.5	.04			

Table 3-16

EXPERIMENT III, FLAGELLATES (x 10^5 μ^3/ml) continued

Days 56-77

	x_i	σ_i	$\bar{x}$	S	R
A	1.03	.07		((G))	
B_1	.15	.02			
2	.30	.04	.23	.07	2.42
3	.23	.03		((P))	(A)
C_1	.5	.07			
2	.13	.02	.61	.54	8.17
3	1.2	.09		((P))	(P)
D_1	-			-	
2	2.48	.13	.83	1.43	18.7
3	-			((P))	(P)
Field	1.88	.14			

Table 3-17

EXPERIMENT III, ULOTHRIX (x 10^6 μ^3/ml)

	Days 56-77					Taken from bottom and Averaged over days 56-77*
	x_i	σ_i	$\bar{x}$	S	R	x_i
A	-			((P))		-
B_1	.07	.04				
2	1.05	.16		((P))		1.27
3	1.68	.30				1.13
C_1	.65	.16				1.9
2						.4
3	.16	.08		((P))		.4
D_1	4.24	.75				1.90
2				((P))		.4
3						

*Error was large. The 95% confidence interval is ±75 X_i -- see Appendix A.

Table 3-18

EXPERIMENT III, TOTAL PHYTOPLANKTON VOLUME (x 10^5 μ^3/ml)

Days	7-14					14-56				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A	12.37	.9005		((E))		1.92	.0825		((G))	
B_1	15.0	1.1				2.77	.1315			
2	11.5	1.0	14.0	2.18	2.04	1.69	.0825	2.13	.57	5.46
3	15.5	1.1		((E))	(G)	1.93	.0922		((G))	(G)
C_1	16.77	1.101				3.89	.1432			
2	12.58	.802	15.67	2.71	2.59	3.79	.1432	4.01	.30	2.19
3	17.65	1.201		((E))	(G)	4.35	.1616		((G))	(G)
D_1	18.11	1.201				3.20	.1118			
2	12.00	.9	15.20	3.07	2.86	1.50	.1140	2.33	1.20	16.08
3	15.50	1.		((E))	(G)	2.30	.12		((F))	(A)
Field	15.5	1.1004				4.1	.1456			

Table 3-19

EXPERIMENT IV, NH_4^+(μM(N))

Days	7-21					21-59				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	2.98	.21				2.54	.132			
2	2.60	.198	4.0	2.2	8.87	2.89	.136	2.9	.3	2.18
3	6.53	.318		((E))	(F)	3.14	.144		((E))	(G)
B_1	4.58	.258				3.11	.144			
2	4.55	.258	4.9	.64	2.38	4.42	.168	4.6	1.6	9.21
3	5.68	.288		((E))	(G)	6.23	.204		((E))	(F)
C_1	3.68	.228				2.44	.128			
2	5.15	.276	4.2	.8	3.24	4.62	.172	3.6	1.1	7.25
3	3.73	.234		((E))	(A)	3.67	.152		((E))	(F)
D_1	3.73	.234				2.31	.128			
2	4.10	.246	3.7	.4	1.72	2.72	.136	2.7	.4	2.97
3	3.25	.216		((E))	(E)	3.03	.14		((E))	(G)
Field	4.15	.24				3.91	.24			

Table 3-20

EXPERIMEMT IV, $NO_2^-+NO_3^-$(μM(N))

Days	7-21					21-59				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	3.35	.462				2.64	.26			
2	2.38	.366	3.0	.5	1.17	15.33	.928	7.0	7.2	12.4
3	3.18	.444		((E))	(E)	3.14	.292		((P))	(P)
B_1	4.68	.60				4.37	.384			
2	2.83	.402	3.6	1.0	2.05	3.77	.34	4.3	.4	1.07
3	3.15	.438		((E))	(F)	4.65	.40		((E))	(E)
C_1	2.88	.408				4.26	.376			
2	10.5	1.05	7.2	3.9	4.73	9.27	.652	5.8	3.0	6.28
3	8.08	.876		((P))	(P)	3.81	.344		((A))	(P)
D_1	7.13	.816				3.13	292			
2	5.48	.672	6.0	.95	1.31	3.20	.296	2.94	.4	1.42
3	5.50	.678		((P))	(E)	2.50	.252		((E))	(E)
Field	2.65	.36				4.73	.44			

Table 3-21

EXPERIMENT IV, DIATOMS (x 10^5 μ^3/ml)

Days	7-21					21-50				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	3.97	.28				.18	.06			
2	.76	.05	2.11	1.66	9.43	.14	.04			
3	1.61	.11		((P))	(P)	.23	.04		((P))	
B_1	2.62	.19				.1	.05			
2	.79	.06	1.62	.93	7.23	.5	.07			
3	1.45	.10		((P))	(A)	.06	.08		((P))	
C_1	.51	.07								
2	1.17	.08	1.18	.67	6.91					
3	1.85	.13		((P))	(A)	.3	.04		((P))	
D_1	2.43	.17								
2	3.55	.25	2.74	.7	3.54	.93	.13	.31	.54	7.15
3	2.25	.16		((P))	(A)				((P))	
Field (0')	2.94	.18			2.98	.16				
Field(12')	3.45	.21			2.23	.12				
	Stephanodiscus					Stephanodiscus				
						Fragilaria				
						Asterionella				

Table 3-22

FLAGELLATED FORMS (x 10^5 μ^3/ml)

Days	7-21					21-50				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	6.03	.43				5.03	.35			
2	10.5	.75	6.27	4.12	8.12	2.44	.24	3.57	1.33	4.77
3	2.27	.16		((P))	(P)	3.24	.23		((P))	(A)
B_1	2.6	.18				4.98	.35			
2	2.8	.20	2.68	.11	.58	1.67	.12	2.88	1.82	7.82
3	2.63	.19		((E))	(E)	2.00	.16		((P))	(A)
C_1	6.08	.43				4.54	.36			
2	10.3	.73	9.71	3.37	4.69	6.34	.44	4.10	2.48	7.48
3	12.75	.91		((P))	(A)	1.43	.08		((P))	(A)
D_1	11.55	.82				5.34	.43			
2	2.65	.19	8.42	5.00	7.55	.86	.07	2.98	2.25	7.98
3	11.05	.78		((P))	(A)	2.74	.22		((P))	(P)
Field (0')	2.3	.23								
Field (12')										

Table 3-23

EXPERIMENT IV, CRYPTOCHRYSIS (x 10^4 μ^3/ml)

Days	7-21					21-50				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	2.85	.36				.23	.08			
2	7.00	.89	4.65	2.13	3.38	.08	.03	.20	.11	1.37
3	4.10	.52		((P))	(A)	.3	.11		((F))	(E)
B_1	2.16	.28				.08	.03			
2	.15	.05	1.27	1.02	4.50	.08	.03			
3	1.5	.27		((F))	(P)				((P))	
C_1	3.5	.63								
2	1.47	.26	2.27	1.08	2.59	.03	.02			
3	1.85	.24		((F))	(A)				((P))	
D_1	2.62	.33								
2	3.35	.43	3.32	.63	1.49	.12	.04			
3	3.85	.49		((F))	(E)				((P))	
Field (0')	1.25	.16				.31	.07			
Field (12')	1.85	.24				.39	.07			

Table 3-24

EXPERIMENT IV, TOTAL PHYTOPLANKTON VOLUME (x 10^5 μ^3/ml)

Days	7-21					21-50				
	x_i	σ_i	$\bar{x}$	S	R	x_i	σ_i	$\bar{x}$	S	R
A_1	12.85	.63				5.44	.36			
2	18.26	1.39	13.03	5.14	5.48	2.66	.25	3.69	1.52	5.17
3	7.98	.56		((F))	(A)	2.97	.26		((G))	(A)
B_1	7.38	.38				5.08	.35			
2	3.74	.21	5.57	1.82	5.72	2.17	.14	3.28	1.57	6.51
3	5.58	.34		((G))	(A)	2.60	.18		((G))	(A)
C_1	10.09	.44				4.54	.36			
2	12.47	.78	13.27	2.86	3.68	6.34	.44	4.10	2.48	7.48
3	16.45	.95		((F))	(G)	2.74	.22		((F))	(P)
C_1	16.60	.90				5.34	.43			
2	9.55	.53	14.43	4.24	5.24	.86	.07	2.98	2.25	7.98
3	17.15	.93		((F))	(A)	2.74	.22		((P))	**(P)**
Field (0')	6.49	.33				3.29	.15			
Field (12')	5.30	.32				2.62	.14			

Table 3-25

EXPERIMENT IV, COPEPODS (x 10^5 μ^3/ml)

On day 50

	x	S	R	
A	2.73	1.89	4.85	((P))
B	4.28	5.88	11.20	((P))
C	3.64	3.12	7.20	((P))
D	2.20	1.59	4.40	((P))

	x	σ
Field (0')	.2	.09
Field (12')	-	

treatment sets for each of the four experiments. As Tables 3-2 through 3-25 show, replication is significantly worse after 56 days, and so the summary Tables cover only the first 56 days of each experiment. For phytoplankton, we only performed this rating for those taxa which dominated, by volume, the particular time period of interest. When only insignificant numbers of a phytoplankton were present, it was excluded from the tabulation. Table 3-26 gives the results of this tabulation for the nutrients NO_3^- + NO_2^- and NH_4^+; while Table 3-27 gives the results for the dominant (by volume) taxa of phytoplankton.

For experiments I - III, nutrient replication was good-excellent. For experiment IV, it was not nearly as good and we can offer no reason why this was so (see Table 3-26). For experiment III, replication of dominant phytoplankton species and total volume data was good. For the other experiments it was generally adequate (see Table 3-27). One plausible reason for this difference is that the phytoplankton numbers initially present in experiment III were an order of magnitude greater than in the other two experiments initiated directly from lake water (II, IV). Experiments II and IV were initiated with lake water containing very low concentrations of phytoplankton (total volume $\sim 10^5$ μ^3/ml) so that small initial fluctuations in phytoplankton numbers per species between replication tanks could induce large errors in replication in subsequent evolution of tanks.

Summary. For 56 days the nutrient replication was good-excellent and the taxa-by-taxa phytoplankton volume replication was adequate to good. Total phytoplankton volume seems to replicate slightly better than individual species data. Whether or not tanks replicated well did not depend on their size or the surface-growth mitigation technique employed. After 56 days, some sets of tanks replicated well, while others did not, with no general pattern emerging as to which would and which would not.

Size Effects

We discuss variations in microcosm behavior as a function of size both for systems where surface-growth mitigation techniques were employed and where they were not. We consider the latter case, not because we believe lake microcosms should be run with surface growth allowed, but because this information will help evaluate the work of other researchers who do not eliminate surface growth.

Table 3-26

SUMMARY OF NUTRIENT REPLICATION OVER FIRST 56 DAYS OF EACH EXPERIMENT

Experiment	Nutrient	Total elements considered	E	G	A	F	P
I							
	NH_3	10	2	7		1	
	$NO_3^-+NO_2^-$	10	8	1			1
II							
	NH_3	12	6	3	2	1	
	$NO_3^-+NO_2^-$	12	12				
III							
	NH_3	8	5	2		1	
	$NO_3^-+NO_2^-$	8	7			1	
IV							
	NH_3	8	1	3	1	3	
	$NO_3^-+NO_2^-$	8	4			1	3

E - excellent

G - good

A - adequate

F - fair

P - poor

Table 3-27

SUMMARY OF PHYTOPLANKTON REPLICATION
OVER FIRST 56 DAYS OF EACH EXPERIMENT

Experiment	Taxon	Total elements considered	E	G	A	P
I						
	Small blue green	5	2		1	2
	Mougeotia	3			2	1
II						
	Fragilaria	3	1		1	1
III						
	Stephanodiscus	6		5	1	
	Flagellate	6	3			3
	Total phyto. vol	6		5	1	
IV						
	Stephanodiscus	4			3	1
	Flagellate	8	1		5	2
	Chryptochrysis	5	2		2	1
	Total phyto. vol.	8		1	6	1

Surface-Growth Mitigation Accomplished by Pouring. As indicated in Table 1-1, experiment I compares 15- and 200-liter systems, II compares 4- and 50- liter systems, while III compares 15-, 50-, and 150-liter systems. We also recall here that experiment I used water which had been in, and conditioned by, the laboratory more than a year prior to initiation while experiments II and III were initiated directly from lake water. Hence, it is reasonable to expect some differences between these two types of experiments.

In all three experiments (I-III), the small poured systems, 15 liters or less, showed significant increases in $NO_3^- + NO_2^-$ by day 63 compared to their larger counterparts. In experiments II and III, these increases were already significant in comparison with the larger systems in the second time interval, namely days 22-56 for experiment II and days 14-56 for experiment III. For these two experiments, linear regressions for each of the replicate system's (E and A respectively) time aggregated $NO_3^- + NO_2^-$ values as functions of time, yield excellent fits to the data (Table 3-28). In summary, the $NO_3^- + NO_2^-$ levels increased steadily from 3.7 μM(N) to 20.7 μM(N) over 74 days in experiment II, and increased steadily from 4.1 μM(N) to 12.4 μM(N) over 67 days in experiment III (Table 3-8 and 3-13). Note that the rate of increase was greater in the 4-liter systems than in the 15-liter systems.

These increases in $NO_3^- + NO_2^-$ in the small poured systems do not seem related to phytoplankton phenomena, as both species and volumes were very different in each of the three experiments. In particular, we note that not only were the species of phytoplankton present different in experiment II than in experiment III, but that throughout each experiment the total volume of phytoplankton was lower ($\sim 10^5$ μ^3/ml) in experiment II than in experiment III ($>10^6$ μ^3/ml) (Tables 3-9 through 3-11 and 3-14 through 3-18). We also recall that the sources of water were different in all three experiments.

The phytoplankton data exhibited some differences between small poured tanks, 15 liters or less, and their larger counterparts, but no systematic behavior (as in the case of $NO_3^- + NO_2^-$) was seen. For the first 56 days of experiment I, there were more Ankistrodesmus and fewer small blue greens in the 15-liter tanks while there were similar levels of Mougeotia in both size classes (Tables 3-4 and 3-6). Throughout experiment II, days 1-92, there were the same major species of phytoplankton present in both the 4- and 50-liter

Table 3-28

LINEAR REGRESSION OF TIME AGGREGATED NO_3^- + NO_2^- DATA ACCORDING TO $y = b + mx$, $y = NO_3^- + NO_2^-$ ($\mu M(N)$), x = time (days)

Experiment	Replicate	Size	b(μM(N))	m(μM(N)/days)	r
II	E1	4	.0386	.250	.996
II	E2	4	-.449	.259	.998
II	E3	4	-1.6	.361	.9985
III	A1	15	1.92	.169	.9627
III	A2	15	3.051	.103	.9923
III	A3	15	1.356	.179	.9853

tanks (systems E and B respectively), but taxa-by-taxa, time interval-by-time interval there were slightly higher levels of phytoplankton in the larger tanks. For the first 56 days of experiment III, the phytoplankton species and levels were similar in the small and large tanks. After day 56, the two size classes diverged. In the 15-liter tanks, Gloeocystis dominated with low levels of flagellates present, while in the 50-liter (B) and 150-liter (D) tanks Ulothrix dominated, with low levels of flagellates present (Tables 3-14 through 3-18).

In experiment III, we compared poured 50-liter systems (B) with poured 150-liter systems (D) (Tables 3-11 through 3-18). For the first 56 days, the nutrient and phytoplankton behavior were quite independent of container size. Diatoms (Stephanodiscus) were the dominant phytoplankton, with low levels of flagellates present also in this time period. After day-56 the replication was not as good as before; although again the nutrient and phytoplankton behavior was similar in both size classes. In particular, the macrophyte, Ulothrix, dominated and low levels of flagellates were present.

Surface-Growth Mitigation by Decanting. In experiment I, an increase in $NO_3^- + NO_2^-$ in the 15-liter decanted systems was not observed, in contrast to the 15-liter poured systems.

Surface-Growth Mitigation by Siphoning. We do not have available size comparisons within the same experiment for this technique.

No Surface-Growth Mitigation. In experiment I, 15-liter systems (E) are compared with 200-liter systems (A), and in experiment II, 4-liter systems (F) are compared with 50-liter systems (C). Unlike the poured situation, there were no systematic differences between the nutrient levels in large and small tanks, but there were significant, non-systematic differences in phytoplankton behavior. During the course of both experiments, the nutrient data was almost identical for large and small systems (Table 3-2, 3-3, 3-7, and 3-8). Both NH_4^+ and $NO_3^- + NO_2^-$ remained at low levels. In experiment I there were significantly more Ankistrodesmus in the 15-liter systems than in the 200-liter systems over the first 56 days. Throughout experiment II, days 1-92, there were the same major species of phytoplankton present in both 4- and 50-liter systems; however, taxa-by-taxa, time interval-by-time-interval, there were significantly higher levels (at least twice as high) than in the larger tanks.

Summary. The results of size-comparison experiments can be summarized as follows:

- Poured systems smaller than or equal to 15 liters showed significant increases in NO_3^- + NO_2^- over their larger poured counterparts by day 63 in all three experiments. This was independent of types or amounts of phytoplankton present and we speculate that it is due to increased bacterial activity. The effect was greater in 4-liter tanks than in 15-liter ones. In the two experiments (II and III) initiated directly from lake water, the increases of NO_3^- + NO_2^- in the small tanks relative to the large tanks were already significant in the second time intervals (days 22-56 for experiment II and days 14-56 for experiment III).

- Both poured and unpoured systems smaller than, or equal to, 15 liters showed different phytoplankton behavior, than their larger poured and unpoured counterparts. These differences, though significant with regard to interpretation of any one experiment showed no systematic pattern from one experiment to another.

- No significant differences were observed between the poured larger tanks (50-liters versus 150-liters); their nutrient and major phytoplankton genera exhibited similar behavior in all time intervals

We observe that the two size classes of microcosms with surface growth eliminated and which behaved similarly, namely 50 and 150 liters, also were of the same depth ~75 cm. On the other hand, the smaller microcosms ($\leq$15 liters) with surface growth eliminated which behaved differently than their larger counterparts were considerably shallower with depths $\leq$25 cm. Thus depth, and not just volume, may be a key parameter in determining size effects in microcosms.

Tracking of Parent Water Body by Microcosms

In each of the three tracking experiments (II-IV), surface water from one of the two lakes (Briones and Lafayette) was used to fill the microcosms. During any year, drawdown on the two lakes was negligible. Water temperature and nutrient input due to run-off were different during each experiment, and are discussed below and in Section 4. Our tracking criteria are discussed in Appendix A; the degree of tracking exhibited by the various microcosm configurations in the light of these criteria is described here.

EXPERIMENT II: Lafayette Reservoir, Nov. 19, 1978 - Jan. 17, 1980. In this experiment the overall differences between the lake and each of the microcosm

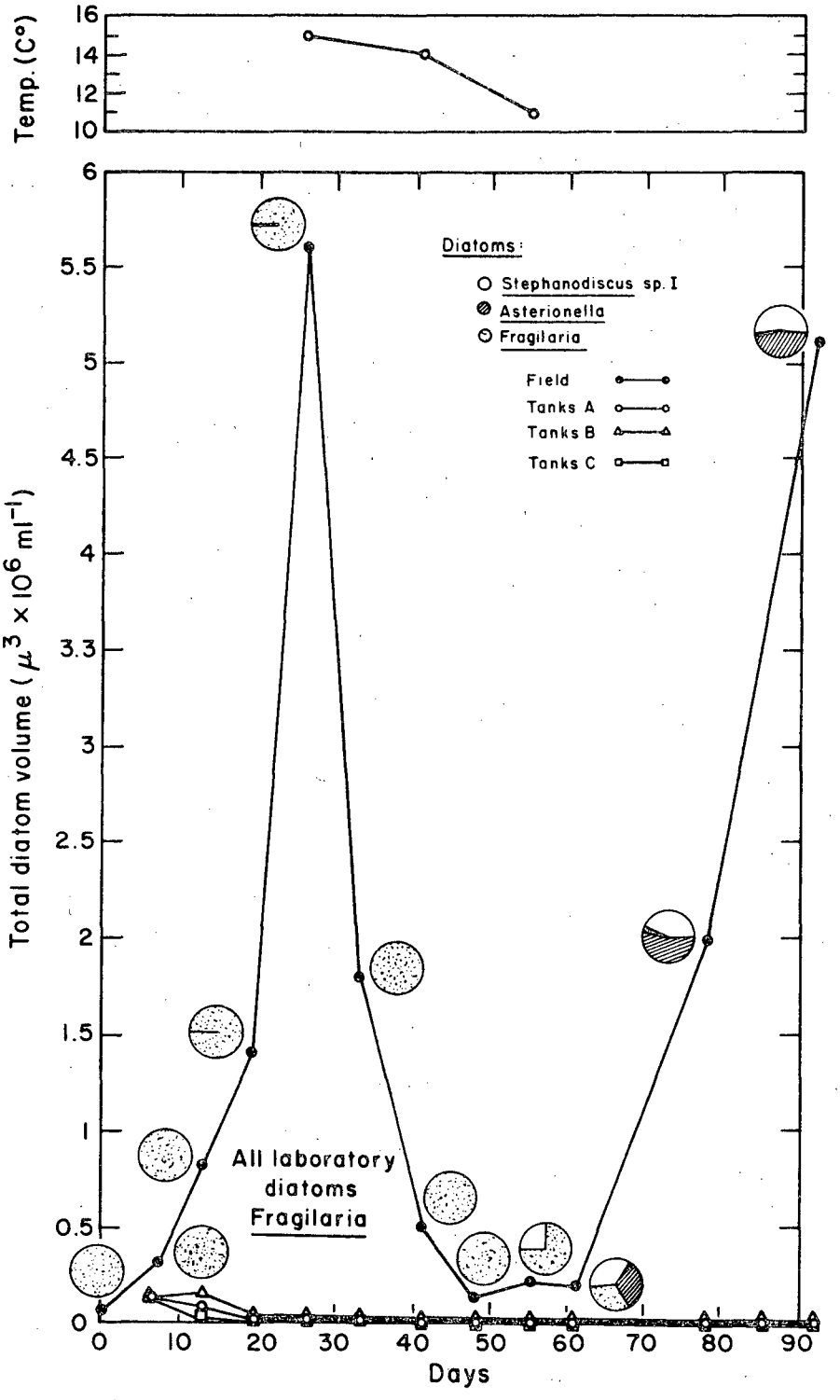

Figure 3-1 Experiment II
(a) Field temperature and (b) Diatom populations for field and laboratory microcosms. Each point of the field data represents the mean of 2 samples. Each point of laboratory data represents the mean of 3 triplicate laboratory microcosms. All laboratory diatoms are Fragilaria. The circles above each field data point represent relative abundances of the designated diatom species. Actual field diatom percentages are given in Table 3-29.

Table 3-29

EXPERIMENT II
ACTUAL PERCENTAGE BY DAY OF DIATOM SPECIES FOUND IN FIELD SAMPLES.
RELATIVE PERCENTAGES REPRESENTED BY PORTION OF CIRCLES ABOVE
FIELD DATA POINTS IN FIG. 4-1

Day	Stephanodiscus Sp. I	Asterionella	Fragilaria
6			100
12			100
19	0.1		99.9
26	1.4		98.6
33			100
41			100
47			100
54	31.0		69.0
61	34.5	31	34.5
77	42.0	51.5	6.5
91	58.3	41.0	0.6

configurations were sufficiently large as to make the individual differences among the various configurations relatively insignificant. Separate comparisons between the lake and each particular configuration thus are not necessary to assess tracking. The experiment ran from fall into the winter. The lake temperature dropped from 16°C to 11°C during the course of the experiment, and was significantly colder than the microcosms which were maintained at 19°C. The lake received high levels of runoff (~35% of the lake's volume) due to heavy rainfall during much of the experiment. Human impact on the lake was large, as it is used for recreation and the watershed includes substantial habitation. The lake's ammonia concentrations reflected the human impacts on the run-off and the large amount of run-off. In particular, NH_4^+ increased from 2.5 μM(N) on day 22 to ~15μM(N) on day-56. From day 56 through day-92 the NH_4^+ levels were still high with a time-aggregated value of 13.41 μM(N) (Table 3-7). The lake's NO_3^- + NO_2^- levels gradually increased from ~2.5 μM(N) to 8 μM(N) during the course of the experiment. The time aggregated NO_3^- + NO_2^- levels varied from 3.2 μM(N) to 5.6 μM(N).

Throughout the experiment, diatoms were the major phytoplankton group in the lake. During days 1-22, Fragilaria was the major diatom present (~6 x 10^5 μ^3/ml); during days 22-56, Fragilaria and Stephanodiscus were both present (total diatom volume ~17 x 10^5 μ^3/ml); and by day-92 Fragilaria, Stephanodiscus, and Asterionella were all present (diatom vol. ~14 x 10^5 μ^3/ml). During days 22-56, low levels of Anabaena were also present (~10^5 μ^3/ml), and during days 56-92 low levels of Ceratium were present (1.7 x 10^5 μ^3/ml) in the lake.

The microcosms' behavior was notably different from that of the lake, as shown in Tables 3-7 through 3-11 and Fig. 3-1. Between days 1 and 22 only low levels of Fragilaria were present in the microcosms (< 10^5 μ^3/ml) while after day 22, no Fragilaria, Stephanodiscus, or Asterionella were seen in the microcosms. Furthermore, in contrast with the lake, no Anabaena or Ceratium were seen in the microcosms after day-22. Between days 56-92, the laboratory systems contained either filamentous greens (~5 x 10^5 μ^3/ml) or flagellates (~10^5 μ^3/ml), while the lake had none of these species in large enough numbers to be noticeable. As will be discussed later, a diatom, Coscinodiscus, not seen in the lake, appeared in some sets of microcosms.

With the exception of the poured 4-liter microcosms (E), the nutrient variations in the microcosms were unremarkable. The time-aggregated values of

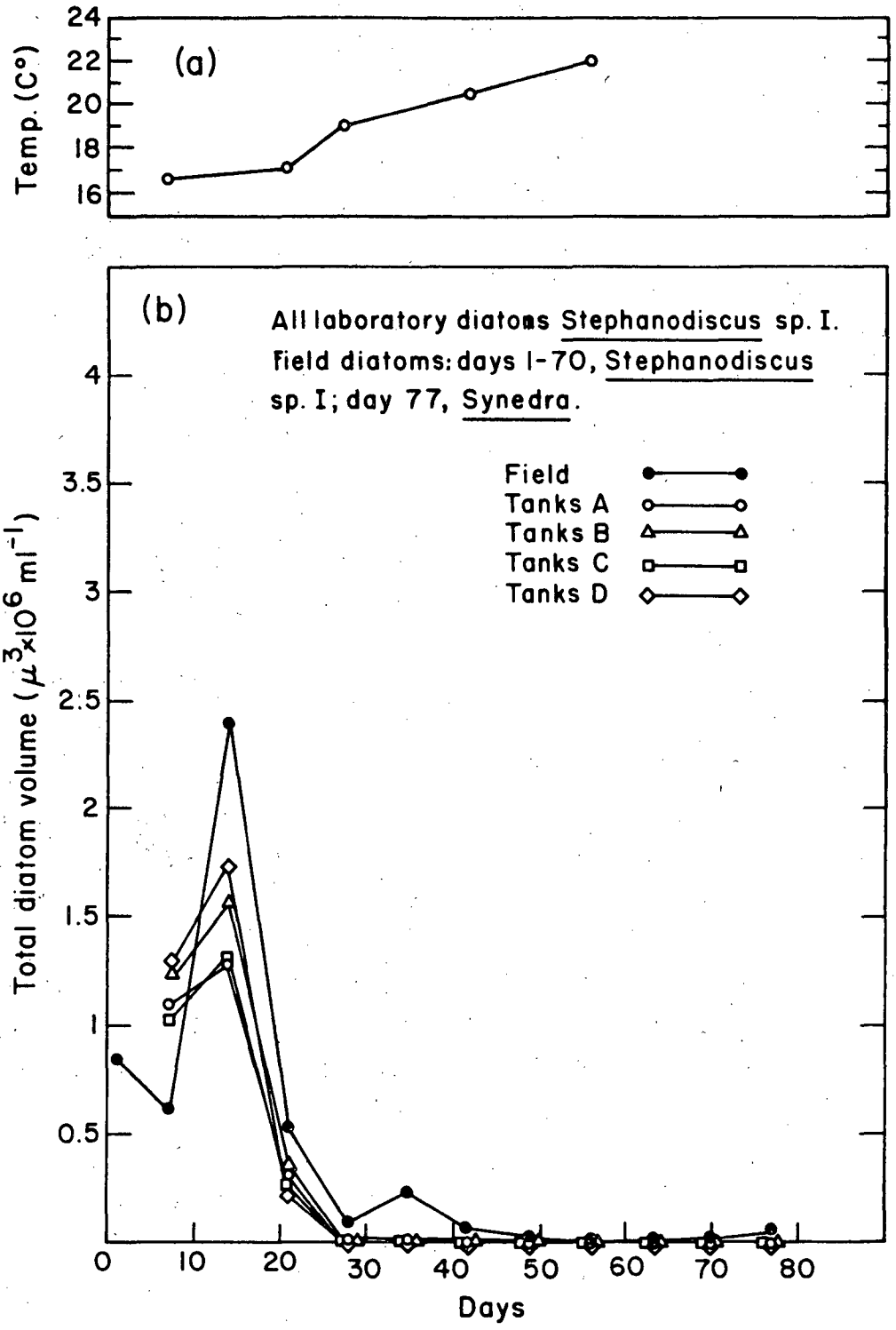

Figure 3-2 Experiment III.
(a) Field temperature and (b) Diatom populations for field and laboratory microcosms. Diatoms in both field and laboratory and all Stephanodiscus. Each point of the field data represents the mean of 2 samples. Each point of laboratory data represents the mean of 3 triplicate laboratory microcosms.

NO_3^- + NO_2^- remained between 3 and 7 μM(N) with no salient patterns apparent. In all the microcosms the time-aggregated NH_4^+ levels were between 2 and 4 μM(N), and thus low compared to the lake.

In summary, we note that physical conditions at the lake (namely, low water temperature and large NH_4^+ input) were significantly different from laboratory conditions. Therefore, the poor tracking was not surprising. The demise of the diatoms after day 22 and the succession to flagellates and/or filamentous greens in the microcosms will be discussed further in the subsection on generic properties.

EXPERIMENT III. Briones Reservoir, April 17, 1979 - July 5, 1979. In this experiment, tracking as a function of size and inoculation was studied. By inoculation, we refer to the addition to a microcosm of a small quantity of water taken from the parent water body at weekly intervals. The sizes studied were: 15-liter (systems A), 50-liter (systems B + C), and 150-liter (systems D). Only Systems (C) were inoculated with lake water (one liter) each week. In all systems surface-growth mitigation was carried out. The experiment ran from late spring into early summer, during which time the lake's surface-water temperature went from 17°C to 21°C. There was less than 5% (by volume) input to the lake during this period. On days 50-77, some of this input came from a eutrophic reservoir, which may account for low levels of Anabaena and Oscillatoria seen in the field samples. On about day 60 blue-stoning of the lake was carried out by the East Bay Municipal Utility District. There was no evidence of noticeable nutrient input to the reservoir's epilimnion during the experimental time period.

In discussing both the lake's and the microcosms' phytoplankton and nutrient behavior, time-aggregated values again will be used in reaching quantitative conclusions. Tables 3-12 through 3-18 and Fig. 3-2 display the relevant information.

During the first 14 days of the experiment, Stephanodiscus was the dominant (by volume) species present in the lake (15.1×10^5 μ^3/ml), with low levels of flagellates also present at densities averaging $.39 \times 10^5$ μ^3/ml. During the next time interval, days 14-56, Stephanodiscus levels decreased to 3.6×10^5 μ^3/ml. Flagellates were still present at low levels ($.32 \times 10^5$ μ^3/ml) and sporadic appearances of individual Ceratium cells occurred.

Stephanodiscus made up 88% of the total phytoplankton volume in the lake during this second time period. Nutrient levels remained low with NH_4^+ going from 5.15 μM(N) during the first time interval to 3.29 μM(N) during the second time interval. NO_3^- + NO_2^- levels also remained low going from 1.65 μM(N) to 1.44 μM(N).

During the first 14 days, Stephanodiscus volume densities in all sets of microcosms were within 20% of the Stephanodiscus volume density in the lake. In both the microcosms and the lake, Stephanodiscus made up more than 97% of the total phytoplankton volume. As in the lake, low levels of flagellates were present in the microcosms.

During the second time interval (days 14-56) both Stephanodiscus and flagellates were present in the microcosms and in the lake. The Stephanodiscus volume densities in the various sets of microcosms, averaged over replicates, ranged from 44% to 76% of the Stephanodiscus volume density in the lake during this period. In the non-inoculated microcosms of all sizes, low levels of flagellates were present. Their volume densities ranged from 31% to 100% of the flagellate volume density found in the lake. In the inoculated 50-liter microcosms (C) the volume density of flagellates was 2.5 times that found in the lake. Individual Ceratium cells were present in the inoculated microcosms on those days they were seen in the lake. During days 14-56, total phytoplankton volume densities in the microcosms were 50% to 100% of the total phytoplankton volume density in the lake.

During the first time interval, NH_4^+ concentration differences between all the microcosms and the lake were small, $\leq$1.3 μM(N), and non-systematic. During the second time interval, NH_4^+ in the small 15-liter systems (A) differed by very little from the lake, <0.4 μM(N). The larger 50-liter and 150-liter microcosms had NH_4^+ concentrations which were slightly higher by .5 to 2.5 μM(N) than the lakes.

During the first interval, the NO_3^- + NO_2^- concentrations in all the microcosms were 1 to 2.5 μM(N) higher than the lakes. As discussed previously, the NO_3^- + NO_2^- concentrations steadily increased in the small 15-liter microcosms and were ~5.3 μM(N) greater than in the lake during the second time period. In the 50-liter and 150-liter microcosms, the NO_3^- + NO_2^- con-

centrations were 1 to 3 μM(N) greater than in the lake during this second time interval.

After 56 days, the lake and the various sets of microcosms diverged considerably. During this period, the dominant phytoplankton in the lake were low densities of flagellates (1.9×10^5 μ^3/ml). Nutrient levels remained low in this time interval with NH_4^+ at 2.95 μM(N) and NO_3^- + NO_2^- at 2.25 μM(N). In the small 15-liter systems (A), high levels of Gloeocystis (10^7 μ^3/ml) were present; NH_4^+ levels were low (2.8 μM(N)) wnile NO_3^- + NO_2^- levels were high (12.4 μM(N)) in this last time interval. In the 50-liter and 150-liter systems (B, C, D), high levels of Ulothrix (10^6 μ^3/ml) were the dominant phytoplankton after day 56; both NH_4^+ and NO_3^- + NO_2^- concentrations ranged between 3 and 7 NM(N).

EXPERIMENT IV: Briones Reservoir, November 20, 1979 - January 11, 1980. The experiment ran from fall through winter. Water temperatures in the lake dropped from 15°C to 11°C during the course of the experiment. Nutrient inputs were not apparent here. Two lake depths were sampled, at the surface and at 4-m depth. Where differences were observed both values are given; otherwise just one value is given. For purposes of tracking, we will compare our microcosms with the surface values since that is where our microcosm water originated. All microcosms were 50-liters in size. The various configurations considered were:

i) no surface growth mitigation (systems A),
ii) siphoning into clean containers to eliminate surface growth (systems B),
iii) pouring to clean containers to eliminate surface growth (systems C),
iv) pouring to clean containers and then back to original container to simulate same mixing levels as in poured systems, but not eliminate surface growth (systems D).

During the first time interval (days 7-21), Stephanodiscus (2.94×10^5 μ^3/ml), Cryptochrysis (1.25×10^5 μ^3/ml), and flagelletes (2.3×10^5 μ^3/ml) were the major species (by volume) in the lake. During the next time interval (days 21-50), the Cryptochrysis ($.39 \times 10^5$ μ^3/ml) and flagellates ($.0 \times$ μ^3/ml) decreased substantially, while diatoms (2.98×10^5 μ^3/ml) continued to be the dominant phytoplankton with Stephanodiscus, Fragilaria, and

Asterionella present. During the first time interval, total phytoplankton volume density was 6.49 x 10^5 μ^3/ml; while the second interval, total phytoplankton volume density was 3.92 x 10^5 μ^3/ml.

Throughout the experiment, the nutrients in the lake remained fairly constant. NH_4^+ levels were 4.15 μM(N) in the first time interval (days 7-21), and 3.91 μM(N) in the second time interval (days 21-59). In the corresponding time intervals, NO_3^- + NO_2^- concentrations were 2.65 μM(N) and 4.73 μM(N) respectively. To contrast behavior of the microcosms with the lake, we make use of Tables 3-19 through 3-24 and Fig. 3-3.

During the first time interval, days 7-21, the major phytoplankton species in the microcosms were the same as in the lake. However, except for the siphoned microcosms (B), the relative densities of the various taxa differed significantly from the field values. In the siphoned systems (B), each major taxa of phytoplankton was at a density within 20% of that in the lake, except for the Stephanodiscus which was at a density about half that in the lake. In the other sets of microcosms, flagellates were 3 to 4 times the density in the lake. During the second time interval, days 21-59, flagellates were the major phytoplankton group in the microcosms (compared with Stephanodiscus, Fragilaria, and Asterionella in the lake). Interestingly, and perhaps coincidently, total phytoplankton volume densities in all sets of microcosms were within ±12 of the corresponding lake value. Diatoms were seen in large numbers throughout the experiment in the reservoir (through day 59), but were not seen after day 29 in the microcosms.

For both time intervals, in the siphoned tanks (B) the concentrations of NH_4^+ and N_3 + NO_2, were within .75 μM(N) of the corresponding lake value. For the other systems, poor replication renders generalizations about their nutrient behavior not useful. Nutrient levels for individual microcosms other than B often differed from the reservoir by > 3 μM(N).

Summary. Our tracking results for all three experiments can be characterized as follows:

- Tracking was best during spring to summer, when the lake's water temperature was closest to the microcosms' and there was little external nutrient input to the lake's epilimnion as in experiment III. Here, good tracking extended over 56 days and was equally

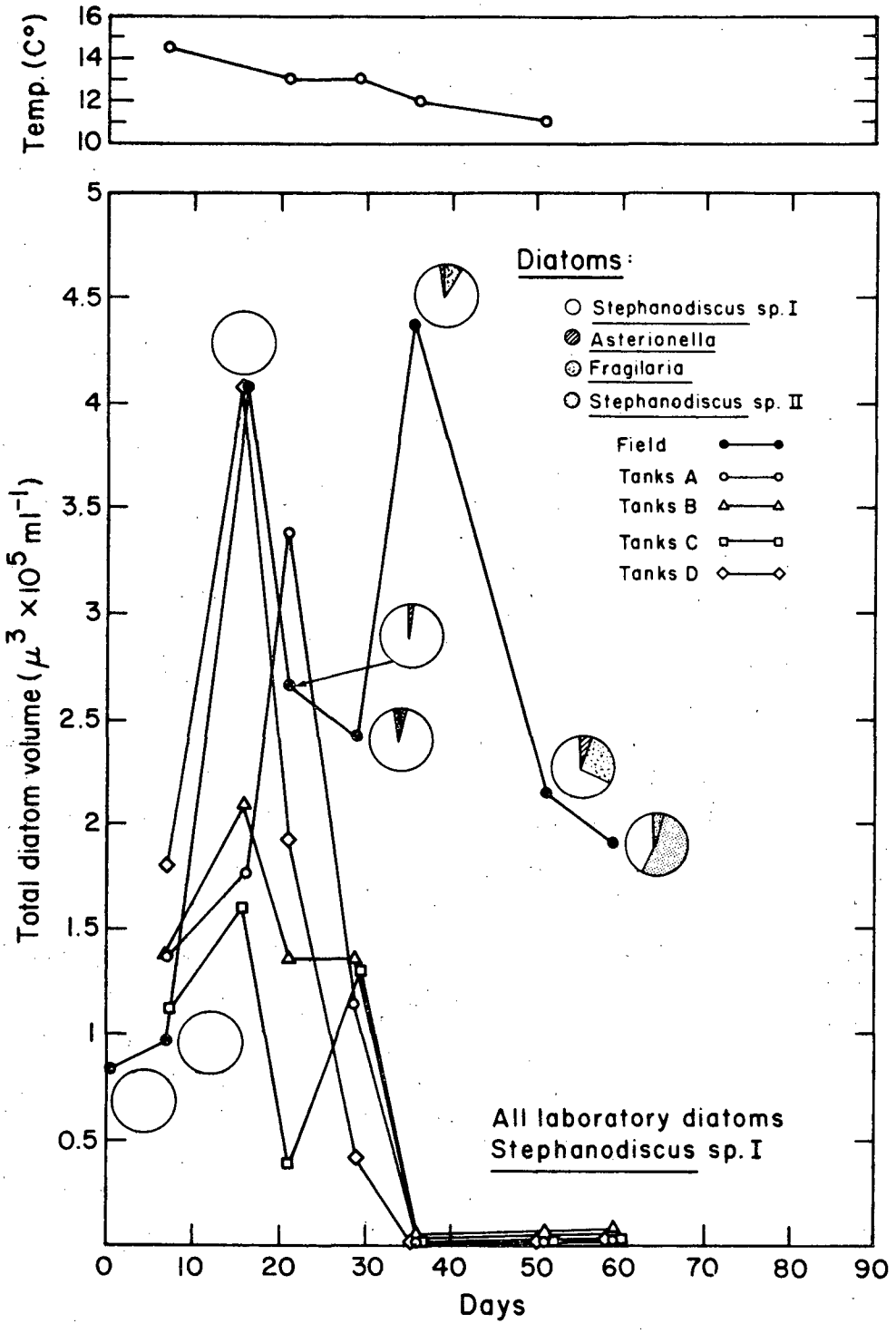

Figure 3-3 Experiment IV
(a) Field temperature and (b) Diatom populations for field and laboratory microcosms. Each point of laboratory data represents the mean of 3 triplicate laboratory microcosms. All laboratory diatoms are Stephanodiscus. The circles above each field data point represent relative abundances of the designated diatom species. Actual field diatom percentages are given in Table 3-30.

Table 3-30

EXPERIMENT IV
ACTUAL PERCENTAGE BY DAY OF DIATOM SPECIES FOUND IN FIELD SAMPLES.
RELATIVE PERCENTAGES REPRESENTED BY PORTION OF CIRCLES ABOVE
FIELD DATA POINTS IN FIG. 3-3

Day	Stephanodiscus sp. I	Asterionella	Fragilaria	Stephanodiscus sp. II
1	100			
7	100			
16	100			
21	98	2		
29	91	3	6	
36	85	1	14	
50	65	7	28	
59	40		7	53

accomplished by 50 and 150 liter poured non-inoculated systems. Throughout the 56 days, both nutrients and major phytoplankton groups in the microcosms exhibited good tracking. In the same experiment, the poured 15-liter microcosms tracked the lake's phytoplankton succession patterns well, but exhibited an increase of NO_3 + NO_2 levels that is characteristic of small, poured systems, as compared with the lake and other microcosms.

- Tracking was next best during fall through winter, when the lake's water temperature was 4-8°C less than the microcosms', and there was no significant nutrient input to the lake's epilimnion as in experiment IV. Here good tracking occurred over 21 days and was accomplished by 50-liter siphoned tanks.

- Tracking was nonexistent during fall through winter, when the lake's temperature was 4-9°C less than the microcosms', and there was significant nutrient input to the lake's eplimnion, as in experiment II.

Agitation

As Perez et al. point out (9) the rate of water agitation can be an important determinant of microcosm behavior. While we intend to carry out a more systematic study of this issue in the future, our experimental design did allow us to examine some differences in behavior induced by different agitation rates, and we report those results here. In experiment II, different agitation rates in 4-liter and 50-liter systems (D,F;A,C) with surface growth can be studied by comparing aerated and non-aerated systems. In experiment IV, different agitation rates in 50-liter systems with and without surface growth can be examined by comparing the non-poured systems (A) with the twice-poured systems (D) and the siphoned systems (B) with the poured systems (C). Below, we mention briefly pertinent observations from these two experiments.

Experiment II. In both size classes with surface growth (4-liter and 50-liter), the less agitated, non-aerated, systems (C,F) had higher levels (>10x) of flagellates than the more agitated, aerated, systems (A,D) (Table 3-13). In the 50-liter systems the less agitated, non-aerated, systems (C) had higher levels of Coscinodiscus (>3 x $10^5 \mu^3$/ml) than the more agitated, aerated, systems (A). The result was reversed in the 4-liter systems with the more agitated, aerated, systems (D) having higher levels of Coscinodiscus (>3 x 10^5 μ^3/ml) than the less agitated, non-aerated, systems (F).

Experiment IV. In 50-liter systems without surface growth, the less agitated, siphoned, systems (B) tracked the parent system better than did the more

agitated, poured, systems (C), showing significantly lower levels of flagellates than did the more agitated poured systems. In the 50-liter systems with surface growth, replication was such that no comparisons can be made.

In summary, we observe that pairs of systems with different levels of agitation but all other parameters identical, showed different phytoplankton succession patterns. These differences, though generally statistically significant with regard to interpretation of each experiment, showed no systematic trends from one experiment to another.

Zooplankton Results

We examined general long-term zooplankton succession patterns in the microcosms that were initiated directly from the lakes, namely experiments II-IV (see Appendix B). Because uncertainties in this data were large, we confine ourselves to obvious observed patterns. By day 70, in all three experiments, the levels of copepods and/or cladocera were at least an order of magnitude higher in the microcosms than in the lake. In Table 3-25, comparison of copepod levels in the microcosms and Briones Reservoir on day 50 of experiment IV illustrate the point. In experiment II, cladocera densities were considerably higher in the microcosms than in the lake by day-13.

EXPERIMENT V

Fluorescence values and phytoplankton volumes for experiment V, in which microcosms were observed for a period of up to 2 years, are presented in Figs. 3-4 and 3-5. In the smaller 15-liter tanks (Fig. 3-4) there was an oscillation of fluorescence values such that observed peak values (averaged over replicates) for both poured and unpoured tanks occurred at the same time of year, on 17 January 1979 and 10 January 1980. A similar situation was seen in the 200-liter tanks (Fig. 3-5) with highest fluorescence values for both poured and unpoured tanks occurring between 11-26 February, and subsequent, more ambiguous, peaks occurring between 18 December and 11 January 1980. Phytoplankton populations and fluorescence values increase in lakes and ponds during summer months when light intensities and temperatures increase. Why fluorescence values of laboratory microcosms exhibited such behavior, despite constant light and temperature conditions, and why this behavior (observed over two years) was about 6-months out of phase with natural systems, remains unexplained.

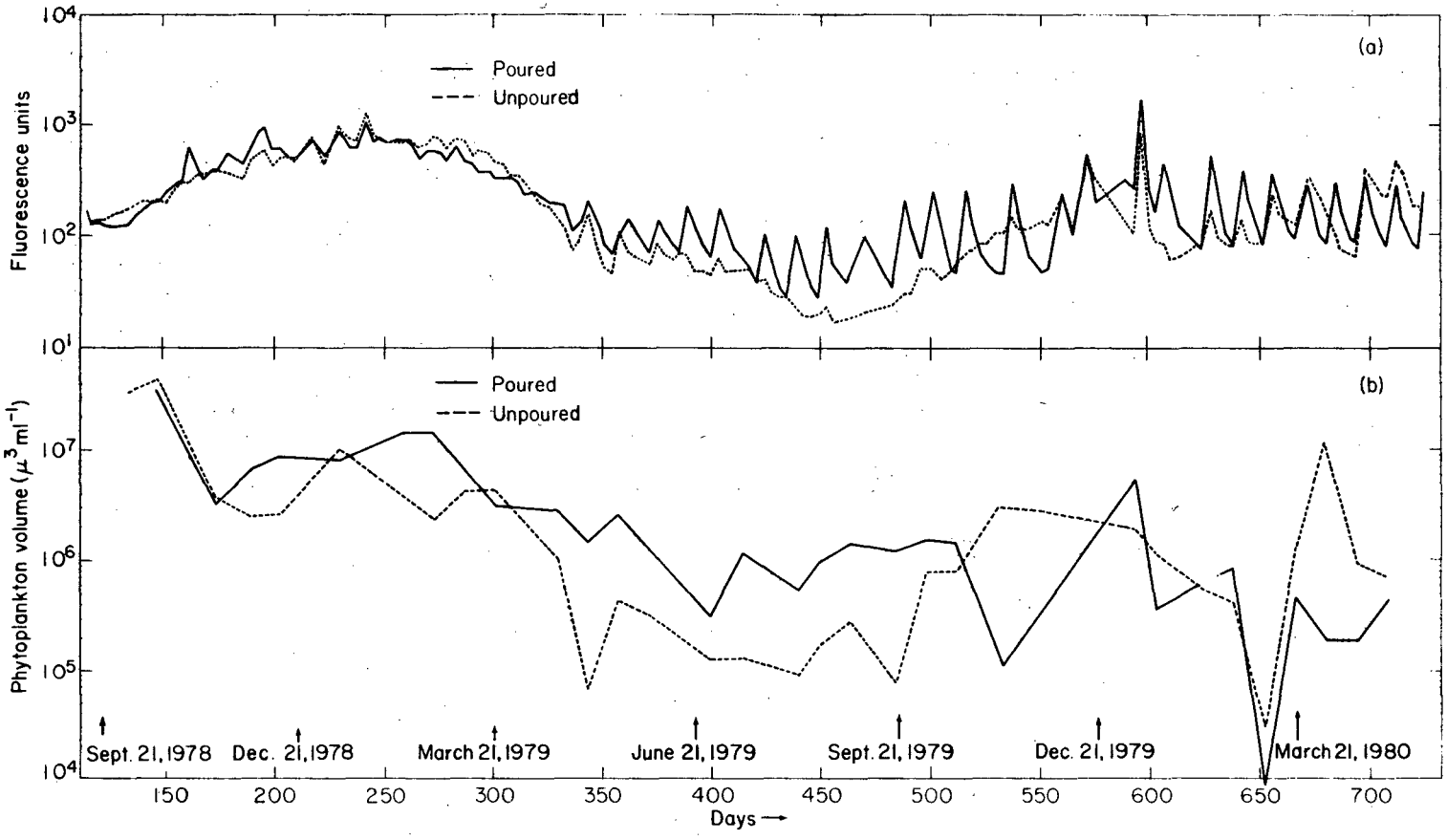

Figure 3-4 Experiment V
(a) Fluorescence values
(b) Phytoplankton concentrations in poured and unpoured 15 liter microcosms monitored for 2 years.
Fluorescence values are the mean of 2 replicates.

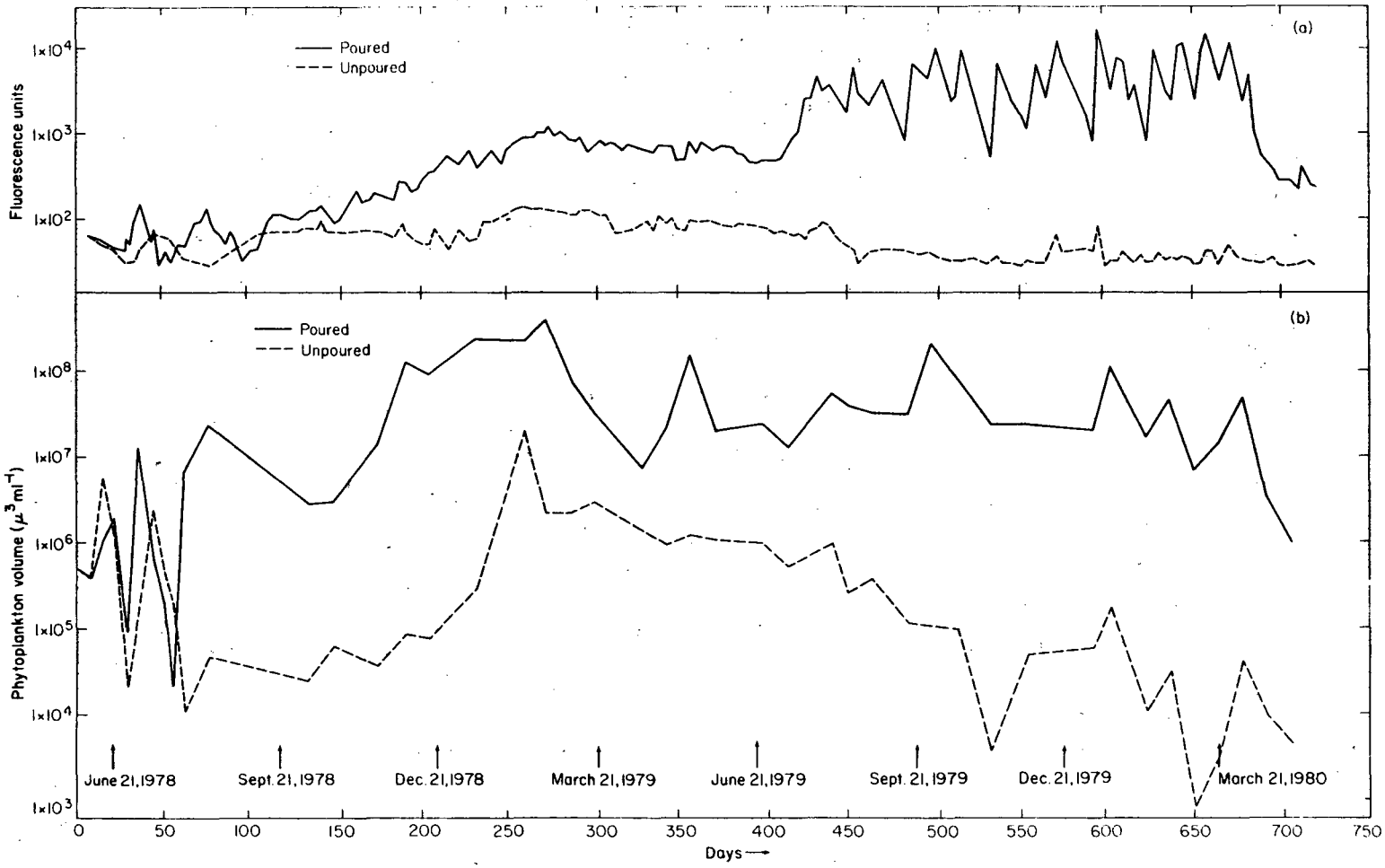

Figure 3-5 Experiment V
(a) Fluorescence values
(b) Phytoplankton concentration in poured and unpoured 200 liter microcosms monitored for 2 years

None of the tanks at the end of the study period had appeared to come to any kind of "steady state" condition. Fluorescence values and phytoplankton and zooplankton volumes were still in a state of flux at this time. Succession of algal genera seemed to be continuing, but no patterns could be ascertained with regard to period of succession (Tables 3-31, 3-32). After the first 4 months of the experiment there were usually only one or two genera of algae present, with generally one species dominating (>85%) by volume. The replicability of the phytoplankton succession pattern in poured and unpoured 15-liter systems can be compared in a qualitative fashion from Table 3-31. In the poured systems, the dominant algal type was the same within a factor of 2 in the two replicates on 18 out of the 33 measurement days; in the unpoured systems, the replicates agreed to within a factor of 2 on 11 out of 33 days. Closterium comprised nearly 100% of the total phytoplankton volume in the poured 200-liter tank between days 415 and 710 (Table 3-32). The increase in fluorescence values in the poured 200-liter tank from days 410 through days 425 was apparently due to a shift to the dominance of Closterium at that time (Fig. 4-5). A similar jump in phytoplankton volume between those dates was apparent.

Poured 200-liter tanks tended to show higher phytoplankton volumes and fluorescence values at a given time than did unpoured tanks, while in the 15-liter systems, the difference between poured and unpoured systems was considerably less marked. Mean fluorescence values for all 15-liter tanks and the two 200-liter tanks appeared similar. The periodic pouring tended to keep algal cells and detrital material in suspension, which was probably chiefly responsible for higher fluorescence and algal counts in the poured tanks. The pouring of the contents from one tank to another every two weeks also resulted in dramatic 2-week oscillations in the poured tanks observable particularly in the poured 15-liter tanks, beginning on about day 350, and in the poured 200 tanks, on about day 450. Loose sediments on the tank bottoms was disturbed during the pouring which probably resulted in increased fluorescence values. Similar but smaller oscillations in the unpoured 15-liter tanks was attributable to replenishing evaporated tank water with distilled water at 2-week intervals, which also disturbed bottom sediments.

In summary, phytoplankton population cycles and successional changes persist in the laboratory over a two year period, under conditions of constant light and temperature. The effect of pouring was considerably greater in the large tanks compared with the small ones, leading to elevated levels of cell volume and

Table 3-31

DOMINANT ALGAE BY VOLUME IN THE UNPOURED (C_1,C_2) AND POURED (D_1,D_2) 15-LITER TANKS OF EXPERIMENT V

Days	C_1				C_2			
		(μ^3/ml)						
133	Closterium	(2.8×10^7)			Closterium	(3.6×10^7)		
147	"	(5.4×10^7)			"	(2.9×10^7)		
173	Ankistrodesmus	(1.9×10^6)			Ankistrodesmus	(5.8×10^6)		
189	"	(2.2×10^5)			"	(4.8×10^6)		
203	"	(1.5×10^6)			"	(3.6×10^6)		
230	"	(7.7×10^6)			"	(1.0×10^7)		
259	"	(2.4×10^6)			"	(3.0×10^6)		
271	"	(2.3×10^6)			"	(2.4×10^6)		
285	"	(3.5×10^6)			"	(4.7×10^6)		
301	"	(3.4×10^6)			"	(4.9×10^6)		
329	"	(2.1×10^5)			"	(1.8×10^6)		
341	"	(7.2×10^4)			"	(5.4×10^4)		
355	"	(1.6×10^5)			"	(6.7×10^5)		
370	"	(1.4×10^5)			"	(3.8×10^5);	Reproductive cell I	(5.1×10^4)
400	"	(8.2×10^4)			"	(1.6×10^5)		
415	"	(1.1×10^5)			"	(1.4×10^5)		
441	"	(1.2×10^5)			"	(4.7×10^4)		
450	"	(1.3×10^5)			"	(1.6×10^5)		
464	"	(2.5×10^5)			Repr. body I	(5.2×10^3)		
485	"	(7.9×10^4)			Ankistrodesmus	(5.3×10^4);	Reproductive body I	(6.8×10^3)
499	Gloeocystis	(1.4×10^6);	Ankistrodesmus	(1.7×10^5)	LRGT 3μ diam.	(5.8×10^3)		
513	"	(1.2×10^6)			Closterium	(7.6×10^4);	Andistrodesmus	(5.3×10^4)
534	"	(5.6×10^6)			Ankistrodesmus	(1.1×10^5)		
555	"	(5.2×10^6);	Ankistrodesmus	(1.6×10^6)	Ankistrodesmus	(6.6×10^4);	Reproductive body I	(9.0×10^3)
593	"	(2.9×10^6);	"	(5.0×10^5)	Reprod. body II	(4.4×10^4)		
604	"	(1.3×10^6);	"	(7.4×10^5)	Reprod. body II	(1.5×10^4)		
625	"	(7.9×10^5);	"	(1.5×10^5)	Ankistrodesmus	(2.4×10^4)		
639	"	(5.9×10^5);	"	(8.3×10^4)	"	(2.7×10^4);	Tetraedron	(5.3×10^3)
653	"	(2.0×10^4);	"	(2.0×10^4)	Phacus	(7.4×10^2);	"	(2.7×10^2)
667	"	(1.2×10^6);	"	(1.7×10^5)	Ankistrodesmus	(6.6×10^2);	"	(4.4×10^2)
681	"	(1.8×10^7)			Gloeocystis	(1.1×10^6)		
695	"	(1.1×10^6);	Ankistrodesmus	(1.4×10^5)	"	(2.7×10^5);	Ankistrodesmus	(1.6×10^5)
709	"	(1.0×10^6);	"	(1.5×10^5)	"	(1.1×10^4);	Tetraedron	(3.5×10^3)
							Ankistrodesmus	(1.8×10^3)

Table 3-31 (continued)

Days	D_1			D_2		
133	Closterium	(3.2×10^7)		Closterium	(3.2×10^7)	
147	"	(4.0×10^7)		"	(2.1×10^7);	Ankistrodesmus (3.2×10^6)
173	Ankistrodesmus	(1.2×10^6)		Ankistrodesmus	(5.4×10^6)	
189	"	(5.1×10^6)		"	(7.9×10^6)	
203	"	(9.5×10^6)		"	(7.9×10^6)	
230	"	(1.0×10^7)		"	(6.0×10^6)	
259	"	(1.1×10^7)		"	(1.6×10^7)	
271	"	(1.1×10^7)		"	(1.6×10^7)	
285	"	(8.4×10^6)		"	(4.9×10^6)	
301	"	(3.1×10^6)		"	(2.9×10^6)	
329	"	(1.3×10^6)		"	(4.0×10^6)	
341	"	(1.2×10^6)		"	(1.6×10^6)	
355	"	(1.6×10^6)		"	(3.2×10^6)	
370	"	(6.6×10^5)		"	(1.4×10^6)	
400	Reproductive cell I	(1.1×10^5)		"	(4.7×10^5)	
415	Closterium	(1.6×10^6)		"	(2.8×10^5)	
441	"	(7.7×10^5)		"	(1.5×10^5)	
450	"	(9.8×10^5)		Closterium	(6.0×10^5)	
464	"	(1.0×10^6)		"	(1.4×10^6)	
485	Reprod. body I	(1.5×10^5)		"	(2.0×10^6)	
499	Closterium	(1.3×10^6)		"	(1.4×10^6)	
513	"	(1.5×10^6)		"	(9.6×10^5)	
534	Reprod. body I	(1.3×10^5)		Reprod. body I	(7.1×10^4)	
555	Closterium	(4.0×10^5)		Closterium	(1.3×10^5)	
593	4 μ diam.	(2.7×10^5)		"	(8.9×10^6)	
604	Closterium	(4.0×10^5)		"	(1.6×10^5)	
625	"	(9.6×10^4)		Reprod. body I	(6.1×10^5);	Closterium (4.1×10^5)
639	"	(4.6×10^5)		Tetraedron	(4.8×10^5);	" (4.2×10^5)
653	"	(1.3×10^3);	Phacus (4.5×10^2)	Closterium	(1.2×10^4);	Tetraedron (2.8×10^3)
667	Closterium	(1.6×10^4);		"	(7.4×10^5)	
681	Gloeocystis	(1.5×10^5);	Closterium (5.8×10^4)	Gloeocystis	(8.0×10^4);	Closterium (2.3×10^4)
695	"	(1.9×10^5);	" (5.4×10^4)	"	(6.8×10^4);	" (8.8×10^3)
709	"	(4.1×10^5);	" (5.8×10^4)	"	(2.6×10^5)	

Table 3-32

DOMINANT ALGAL GENERA BY VOLUME IN 200-LITER TANKS OF EXPERIMENT V

Day	Poured (A)	Not Poured (B)
1	Small, unidentified blue-green or bacterium	Small, unidentified blue-green or bacterium
8	Small, unidentified blue-green or bacterium	Small, unidentified blue-green or bacterium
15	Small, unidentified blue-green or bacterium	Small, unidentified blue-green or bacterium
29	Mougeotia	Synedra
36	Mougeotia	Mougeotia
43	Mougeotia	Mougeotia
63	Closterium	Anabaena
77	Closterium	Phacus
133	Schroderia	Anabaena
147	Schroderia	Phacus
173	Anabaena	Phacus
230	Anabaena	Cryptochrysis
259	Anabaena	Chryptochrysis
402	Anabaena	Cryptochrysis
415	Closterium	Cryptochrysis
441	Closterium	Unidentified flagellate
450	Closterium	Schroderia
464	Closterium	Schroderia
513	Closterium	Unidentified flagellate
534	Closterium	Phacus
555	Closterium	Closterium
709	Closterium	Closterium

fluorescence. In the second year of the study, the pattern of phytoplankton succession replicated better in the 15-liter poured tanks than in the 15-liter unpoured tanks, suggesting that the pouring technique is desirable for long-term microcosm studies.

Section 4

MICROCOSM ASSESSMENT STUDIES: DISCUSSION OF RESULTS

In this section we discuss some aspects of our results that are most relevant to potential applications of pelagic microcosm.

Photoplankton Succession. We examined general long term phytoplankton succession patterns in the microcosms that were initiated directly from natural water bodies (experiments II-IV). In all three experiments, diatoms were initially the major types of phytoplankton in the natural water bodies and the microcosms. By day 56, in all three experiments, flagellates, filamentous greens, or green algae were the dominant type of phytoplankton in the microcosms, in contrast to the parent lake. In particular, in experiments II and IV diatoms were dominant after day 56 in the lakes, whereas flagellates and filamentous greens were dominant in the microcosms. In experiment III, low levels of flagellates ($\sim 10^5$ μ^3/ml) were dominant in the lake after day 56, while high levels ($>10 \times 10^6$ μ^3/ml) of filamentous green algae and planktonic green algae were dominant in the microcosms (Figs. 3-1 through 3-3).

At least four different factors that can influence the succession of phytoplankton from diatoms to non-diatoms can be identified (16-20). These are light levels, water temperature, nutrient availability (including silica), and grazing pressure. Many of these factors were different from one tracking experiment to another between the lake and the microcosms. Experiments II and IV were carried out in the late fall and early winter whereas experiment III was carried out in the late spring and early summer. Light levels and temperatures in the lakes were thus lower during experiments II and IV than during experiment III. The light and temperature levels in the microcosms remained nearly constant throughout all experiments. Moreover, seasonal differences in the influx of silica and other nutrients to the surface waters of the lakes during the course of the experiments can be expected (17,18). We know, for instance, that watershed runoff brought a significant amount of inorganic nitrogen to the lake in experiment II. Finally, zooplankton grazing

pressure may have been significantly different in the three experiments. Zooplankton densities generally were higher in the microcosms than in the lakes, particularly in the later time intervals of each experiment.

In experiment II, the diatom population in the lake started out at a relatively low level ($\sim 10^5$ μ^3/ml), underwent a nearly 100-fold increase within four weeks, and then fell back to roughly the initial density by the end of the experiment. Diatoms in the microcosms crashed within a few weeks. In experiment IV, where diatom tracking was also poor, the lake diatom population stayed roughly constant and the microcosm population declined rapidly following initiation of the tanks. In experiment III, initiated in the spring, the diatom population started out an order-of-magnitude higher than in the other two tracking experiments and declined over several orders-of-magnitude in both the lake and microcosms during the first 5 weeks.

The following speculations about the causes of these differences observed in the three tracking experiments are based on admittedly limited evidence. The immediate decline of the large diatom population in the lake in experiment III was likely due, at least in part, to silica depletion in the lake water (17,18). Because this experiment was run during a period in which the lake was stratified and little runoff or other input occurred, silica replenishment was probably negligible, althogh silica concentrations were not measured. Increasing lake-water temperature during the run might have hastened this decline, although data about temperature dependence of the growth rate of the dominant diatoms under the nutrient and light conditions present in this experiment are lacking. Increasing water temperature might also have caused the observed increased zooplankton densities (see Appendix B) and hence grazing pressure in the lake during this period. The temperature of the microcosms during this experiment was close to that of the lake, and this is likely to have increased the degree of tracking observed in this run, relative to that in experiments II and IV where significant temperature differences existed between the microcosms and the parent lakes.

In experiment II, the diatom bloom could well have resulted from the large nutrient additions to the lake at that time. In experiment IV, the low levels of diatoms observed in the lake throughout the late fall and early winter were probably able to sustain themselves on the natural additions of silica and

other nutrients typical of that season. Grazing pressure in the parent lakes was relatively low during that period. The relative decline of diatoms in the microcosms during all three tracking experiments likely was enhanced by a combination of silica depletion and grazing pressure, as there was no input of silica to the tanks and zooplankton densities were higher than in the lakes. The higher temperatures in the microcosms relative to the lakes in experiments II and IV could have worsened the tracking (16); information on the actual magnitude, and even sign, of the effect on diatom growth of higher temperatures under the environmental conditions of these experiments is lacking. The light levels in the microcosm facility were considerably lower than on the surfaces of the lakes and this may also have enhanced the decline of the diatom population relative to that of other phytoplankton in the laboratory (16,19).

Our microcosms are designed to be models of the epilimnion of a stratified lake. They include no mechanisms of nutrient input to simulate those from either the surrounding watershed or from the lake bottom. Thus it is gratifying that the experiment in which phytoplankton dynamics tracked well was experiment III, in which the macroscopic conditions we were trying to simulate in our microcosms were most nearly seen in the natural lake. We note that microcosms could be used to conduct further tracking studies in which factors including light, temperature, silica levels, and the density of grazers are manipulated so as to provide insight into the reasons why unrealistic diatom succession patterns sometimes occur in microcosms.

Diatom Peculiarities

In the experiments (II-IV), where the microcosms were initiated directly from natural water bodies, the diatoms initially present in the microcosms experienced morphological changes not seen in the lake. In particular, diatoms in the microcosms formed microspores which are believed to be a mode of sexual reproduction (21). We do not know what conditions in the microcosms trigger this phenomenon.

The subsequent appearance of different diatom genera in the microcosms after the initial time intervals depended on relative decreased water agitation. In particular, in experiment I, high levels ($\sim 10^5$ μ^3/ml) of Synedra radians were seen during days 21-56 in those microcosms which were not poured as compared to those which were poured or decanted. In experiment II, during days

56-92, high levels (> 3 x 10^5 μ^3/ml) of Coscinodiscus were seen in the non-aerated, non-poured 50-liter microcosms as compared with the aerated microcosms.

Zooplankton Results

Zooplankton densities in the microcosms rose over several months to levels considerably higher than those in the lakes. There are two possible explanations for these observations. First, day-time downward migration patterns of zooplankton eliminated them from surface samples of the natural water body. To examine this possibility, we made vertical profiles of zooplankton in Briones reservoir. Preliminary data indicate that there were up to ten times the number of larger zooplankton at the metalimnion than at the surface. In particular, on November 4, 1979 at the surface there were zero Daphnia seen while at a depth of 12 meters there were 17 Daphnia seen in a two-liter sample. A second possibility is that grazing by fish keeps the larger zooplankton populations low in the lake compared with the microcosms.

Section 5

MICROCOSM INVESTIGATIONS OF DECOMPOSITION ACTIVITY

INTRODUCTION

The experiments described here are quite distinct from the tracking and replication studies presented in the preceeding sections. Our objective here was to carry out an exploratory study of the feasibility of using a relatively simple microcosm technique to determine properties of microbial decomposition in freshwater ecosystems. The longer-term goal, to be reached in planned follow-up research, is to apply this technique to the development of a standardized protocol for measuring impacts of toxic substances on aquatic mineralization and decomposition rates.

Our experimental approach was to add a range of amounts of dead organic matter to lake-water aliquots and then measure the subsequent chemical and biological responses of the system. The anticipated pattern of response to such detritus additions is an increase in inorganic nutrients (mineralization) and microbial uptake of nutrient (immobilization), followed by assimilation of these nutrients by phytoplankton. Such responses can be expected to occur within a few days of the initial detritus addition. Over a longer time period, increased grazing by zooplankton on the enlarged phytoplankton population and increased rate of return of organic matter to the detritus pool can be expected. In the experiments reported here we followed in detail the short-term responses. As described below, we found that the quantitative pattern of response is sufficiently complex and interesting that it provides a detailed characterization of the microbial-detritus dynamics, and in particular is likely to yield useful information about microbial carrying capacities and mineralization rates.

Mineralization, or the production of inorganic nutrients from organic detritus, is an end stage of decomposition. Mineralized inorganic nutrients are potentially available for primary production, but they also can be immobilized for growth of the organisms that carry out decomposition, and they can be exported

from the system as, for example, in denitrification. We define net mineralization to be total mineralization minus immobilization and export. It is thus a measure of the production of inorganic nutrients that are available for primary production. Our interest here was in the relation between net mineralization subsequent to a detritus addition and the size of that addition. Specifically, we were concerned with the production of NH_4^+, NO_2^- + NO_3^-, and CO_2. We added different amounts of identical natural detritus to each member of a set of initially identical lake-water aliquots and determined the dependence of the net quantity of inorganic nitrogen (IN) and inorganic carbon that was mineralized on the amount of detritus that was added.

If the net percentage of substrate that is mineralized up to time t is plotted as a function of t, for times subsequent to the addition of detritus, and if several different concentrations of added detritus are compared in this fashion, then at any given time one might observe that this percentage decreases, stays constant, or increases with increasing initial detritus concentration. Moreover, the dependence of this percentage on initial detritus concentration may vary with the time, t, that has elapsed since the addition of detritus. In the most careful study of this to date, Williams and Gray (22) added small quantities of ^{14}C-labeled amino acids (0.1 µg/liter) and at the same time a range of larger quantities of unlabeled amino acids (100-5000 µg/liter) to sea-water aliquots and observed the resulting respiration rates over a 2-day period. They deduced the following conclusions:

i) Initially, within a few hours after the addition of substrate, the percentage of substrate respired decreased with increasing initial substrate concentration;
ii) The larger the initial substrate concentration, the later the induction time (time of maximum respiration rate);
iii) By the end of 2 days, all systems had respired 30-50% of the added substrate, independent of the amount added. A simple mathematical explanation of these conclusions in terms of Michaelis-Menten uptake kinetics was given.

In related studies with labeled assemblages of amino acids Williams (23) and Hobbie and Crawford (24) have observed that a large fraction of decomposed substrate is incorporated into heterotrophic biomass growth (primarily bacteria). This fraction averages well over 0.5, in contrast with the much smaller incorporation fraction when the added substrate consists of a single detrital

component such as glucose. Given that a large percentage of added substrate is incorporated, it follows that the net mineralization of inorganic nutrients can be quite sensitive to factors influencing the growth of heterotrophic populations. In particular, if density-dependent regulation of heterotrophic biomass growth limits immobilization or incorporation of nutrients for that growth above a certain threshold concentration of added substrate, but does not limit mineralization activity, then net mineralization should account for a larger percentage of added substrate above that threshold than below. This would be in contradiction with deduction (iii) of Williams and Gray (22) discussed above.

However, if one looks at the data of Williams and Gray, it appears that this third conclusion may have been drawn prematurely. In two of the experiments they reported, early termination before the respiration rates had levelled off makes it difficult to reach any conclusion about asymptotic mineralization (their figures 2 and 3) while in the third reported experiment with amino acid mixtures (their figure 1) the data are manifestly at variance with the simple kinetic model used. Again, early termination of the experiment makes it difficult to reach a firm conclusion, but there is evidence from their data that the asymptotic fraction respired is dependent on the amount of substrate added initially.

We hypothesize that the fraction respired is, indeed, dependent on the amount of substrate added and that the dependence is of a threshold nature, with the amount of net-mineralization occurring increasing sharply above a threshold concentration of added substrate. The experiments we report here were designed to test this hypothesis. Our approach differed from that of Williams and Gray in several respects. First, we investigated freshwater lakes rather than marine systems. Secondly, the substrate we added consists of dissolved and particulate fractions of freshly grown, killed, and sterilized freshwater organisms, rather than prepared assemblages of amino acids. Thirdly, we measured mineralization activity over a five-day period or longer following the addition of substrate, thus allowing opportunity to observe mineralized inorganic nutrients reach their maximum levels. Finally, we did not use ^{14}C-labeling here. Our reason for this was the perceived difficulty in obtaining large quantities of uniformly-labeled, freshly-grown and prepared detritus. Since we completed our investigations, a paper appeared by Cole and Likens (25) that describes a method for carrying out decomposition studies with

detritus consisting of ^{14}C-labeled algae. While their study was restricted to considerably smaller fractional increases in detritus concentration than in ours, future application of their method to the problem at hand is intended.

In order to quantify the short-term mineralization activity in our systems, we measured daily water-column concentrations of inorganic nitrogen (NH_4^+ and $NO_2^- + NO_3^-$) over the 5 to 10 days subsequent to the addition of detritus. This time period was usually sufficient to detect a rise and then a fall in the inorganic nitrogen levels. Such measurements, alone, do not allow a separation of inorganic-nitrogen production from inorganic-nitrogen uptake by phytoplankton, and for that reason we made a number of supplementary measurements, including dark- and light-bottle CO_2 evolution and phytoplankton counts. In a variety of natural circumstances neither C nor N is a limiting nutrient; nevertheless, measurements of the type reported here provide information about the mineralization process itself, if not about the potential for enhanced primary productivity subsequent to an addition of detritus.

METHODS

The experiments reported here were carried out in 4-liter glass beakers housed in a temperature-controlled room at 19 ± 1°C. Illumination was provided by a bank of eight 1.3m very high-output, cool-white fluorescent lights on a 12h:12h light:dark cycle; the light irradiance on the water surface of the microcosms was 7.0 ± .3 watts/m^2 PAR. The water in each beaker was agitated gently by air pumped at a rate of about 1 liter per minute through a capillary tube extending 15 cm below the water surface.

Each of the four experiments was carried out with water samples taken originally from lakes in the San Francisco Bay area. Except for experiments K-3 and K-4, which were conducted simultaneously on identical lake water samples, the experiments were carried out sequentially and with different lakes as a source of water. Prior to each experiment, the lake water samples were maintained in large laboratory microcosms (50-700 liters) for a period of several months, where they served as controls for other experiments we were conducting. Because the experiments reported here were performed with lake water samples housed temporarily in laboratory microcosms, it is possible that our results reflect laboratory conditions.

Table 1-2 summarizes the conditions of each of the experiments carried out (labeled K-1 to K-4). In each experiment, the replicate 4-liter beaker systems were initiated from the larger laboratory microcosms three days prior to the addition of detritus, and background values of all monitored quantities were then determined. On day-zero of each experiment, organic carbon was measured in all 4-liter systems and in the concentrated detritus spike. The detritus was then immediately added to all treatment systems, at relative concentrations shown in Table 1-2.

The detritus was prepared in several different ways, depending on the experiment. In two of the experiments, K-1 and K-2, E. coli grown specifically for the purpose were used. These dense cultures reached concentrations of 5 mM (C) (5 millimoles of carbon per liter of water). The E. coli were harvested, sonicated for 30 minutes effectively breaking cell walls, and then autoclaved for 40 minutes at 110°C and 25 psi. To prepare detritus for the other two experiments, algae consisting primarily of Scenedesmus, Chlorella, Gleocystis, Ankistrodesmus, and unidentified small, round, green nanoplankton were grown under nutrient-rich conditions, harvested, and then sonicated and autoclaved. For one of these experiments (K-3), the fine-particle and soluble portion of the algal detritus was separated and used for the detritus addition. Separation was accomplished by first passing the algal culture through a 5μ filter and then by letting the filtrate settle for 24 hours and decanting the top quarter of the filtrate. Hereafter, for the sake of brevity, we denote this portion of the detritus as dissolved organic matter. Immediately prior to the addition of the detritus, its sterility was examined by standard bacterial plating methods (26). No bacterial colonies were observed following the inoculation. Because our systems, like natural lakes, are exposed to the atmosphere, sterile controls were not maintained. Having determined the sterility of the detritus, however, we used replicate systems to which no detritus was added as controls.

With the exception of water-column phytoplankton and zooplankton (number and volume), which were measured approximately weekly, monitoring was carried out daily for periods ranging from one to several weeks. Measurements were made from water samples taken from the 4-liter systems at approximately 4 hours after the onset of light each morning, at 11:00h. Integrated water-column samples for measurement were taken with a hollow polyethylene tube (1 cm i.d.) inserted to within 0.5 cm of the bottom of the beaker, stoppered at the top,

and removed. For the sealed-bottle CO_2 and NH_4^+ evolution measurements, 50 ml bottles were used. Table 5-1 lists the methods used for monitoring chemical and biotic parameters.

RESULTS

The four experiments were similar in design while different in initial parameters. Different water samples were used in K-1 and K-2 and K-3, 4; the biological materials from which the added detritus was obtained in K-1, 2 differed from that in K-3, 4; and the size spectrum of the added detritus in K-3 differed from that in K-4. Therefore, identical behavior in the four experiments cannot be expected and, as discussed below, was not observed.

For the sake of clarity, some of the data presented in the accompanying figures (Fig. 5-1 to 5-10) are averaged over replicates rather than displayed separately for each replicate system, but, except for K-4, where measurements in replicate systems were carried out they agreed to within 20% of one another. The replication in nutrient data among the duplicate or triplicate subsystems in experiments K-1 and K-2 was particularly good, as seen in Figs. 6-1 and 6-8.

Table 5-2 lists the organisms other than bacteria present in the 4-liter beakers in K-1. The species list was not identical to this in the other experiments, as expected since their source of water was different. Nevertheless, the variations were not great, with about 80% of these species present in the other experiments. The numbers of these species varied considerably from one experiment to another, as well as during the course of each experiment. In K-1, for example, a ciliate protozoan dominated (by volume) the animal population, while in K-2, a rotifer (Lecane sp.) and a cladoceran (Alona guttata) dominated. In K-3 and K-4, the dominant phytoplankton were Mougeotia sp. and Phacus sp., while in K-1, an unidentified flagellate dominated.

We describe the results of K-1 below in considerable detail and then point out more briefly similarities and differences in the results of K-2, 3, and 4.

K-1. Three levels of bacterially-derived detritus, corresponding to 117, 235, and 470 μM(C) organic carbon, were added to systems B, C, and C respectively.

Table 5-1

METHODS USED FOR MEASURING CHEMICAL AND BIOLOGICAL PARAMETERS

Parameter	Method	Special Equipment	Reference
O_2	polarography	O_2 meter YSL 57)	--
pH	electrometry	pH meter (Orion)	--
IC	infrared absorbance	IR analyzer (Beckman 865)	--
OC	combustion to IC	TOC analyzer (Beckman 915A)	--
NH_4^+	blue indophenol	spectrophotometer (Zeiss PM2 DL)	(10)
$NO_3^-+NO_2^-$	reduction, diazotization	"	(11)
CO_2 evolution	equilibria kinetics	pH meter (Orion 601) IR analyzer (Beckman 865)	(12)
phytoplankton	tube chamber	5 ml tube chamber (Wilde) inverted microscope (Lietz)	--
zooplankton	counting chamber	100 ml count. chamber (Wild) binocular microscope (Lietz)	--

Table 5-2

LIST OF ORGANISMS PRESENT IN THE 4-LITER BEAKERS FOR K-1

CHLOROPHYTA

- Ankistrodesmus sp.
- Chodatella quadrisets
- Closterium sp.
- Mougeotia sp.
- Rhizoclonium sp.
- LRGT I (5)
- LRGT II (5)
- Nephrocytium sp.
- Gloeocystis sp.
- Planktosphaera gelatinosa
- Quadrigula sp.
- Scenedesmus bijuga
- Scenedesmus quadracauda
- Schroderia setigera
- Staurastrum sp.
- Treubaria trippendicular

BACILLARIOPHYCEAE

- Coscinodiscus lacustris
- Cyclotella menenghiana
- Fragilaria sp.
- Navicula sp.
- Synedra radians
- Synedra ulna
- Anomoeneis sp.
- Gomphonema sp.

CYANOPHYTA

- Anabaena sp.
- Oscillatoria sp.
- Spirulina sp.

CYPTOPHYCEAE

- Cryptochrysis sp.

EUGLENOPHYTA

- Phacus sp.
- Unid. flag. I
- Unid. flag. II

PYRROPHYTA

- Unid. Dinoflagellate I

PROTOZOA

- Paramecium sp.
- Vorticella sp.
- Actinosphaerum sp.
- Monas sp.

ROTIFERA

- Ascomorpha sp.
- Discranophorus.sp.
- Keratella quadrata
- Lecane sp.
- Philodina sp.
- Polyarthra sp.
- Trichotria sp.
- Voronkowia sp.
- Unid. rotifer I

ANNELIDA

- Pristina sp.

CLADOCERA

- Daphnia pulex
- Simocephalus vetulus
- Alona guttata

COPEPODA

OSTRACODA

- Cypridopsis sp.

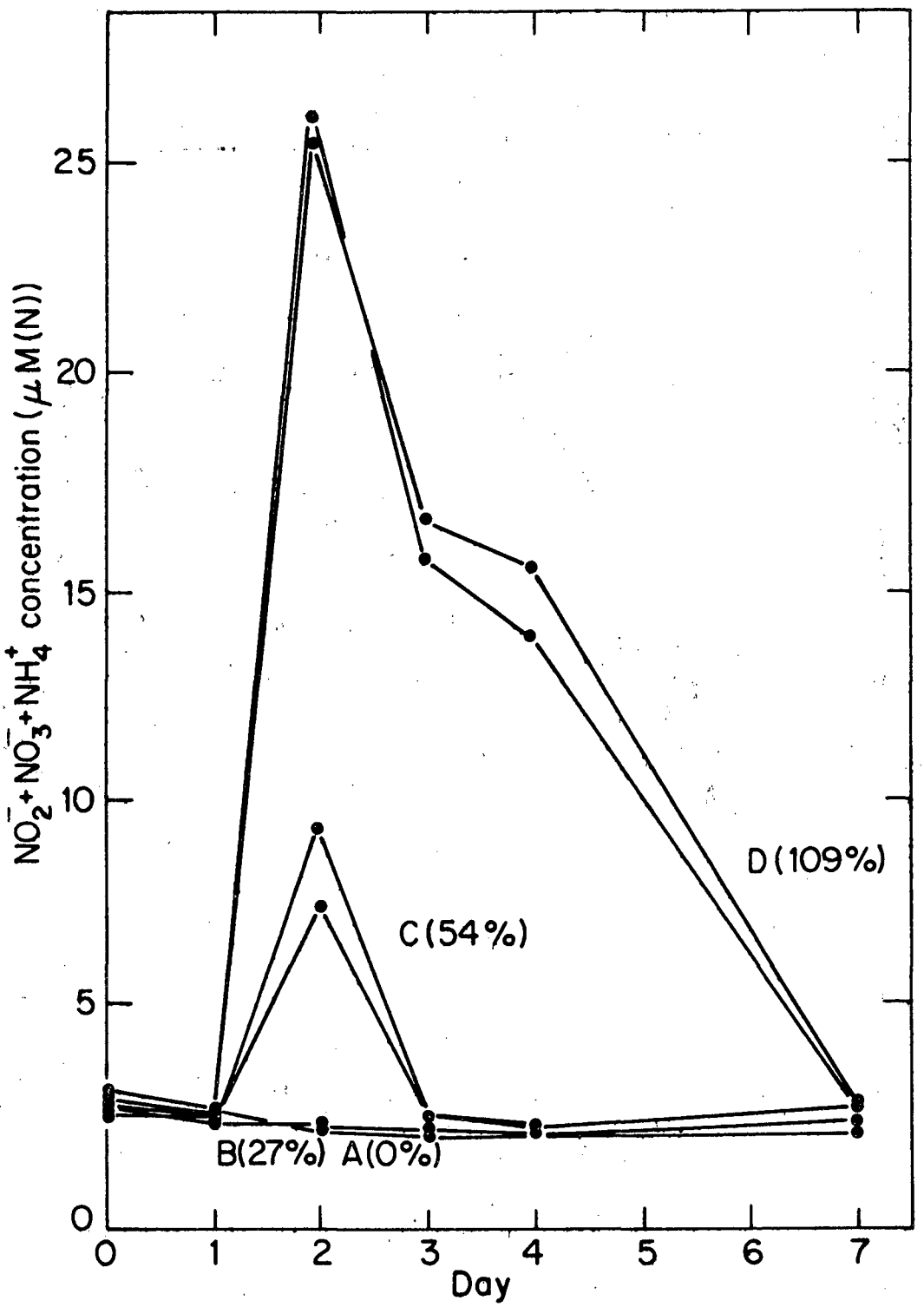

Figure 5-1 $NH_4^+ + NO_2^- + NO_3^-$ concentrations in the treatment (B, C, and D) and the control (A) systems in experiment K-1. Shown in parentheses next to each system label is the percent increase in organic carbon.

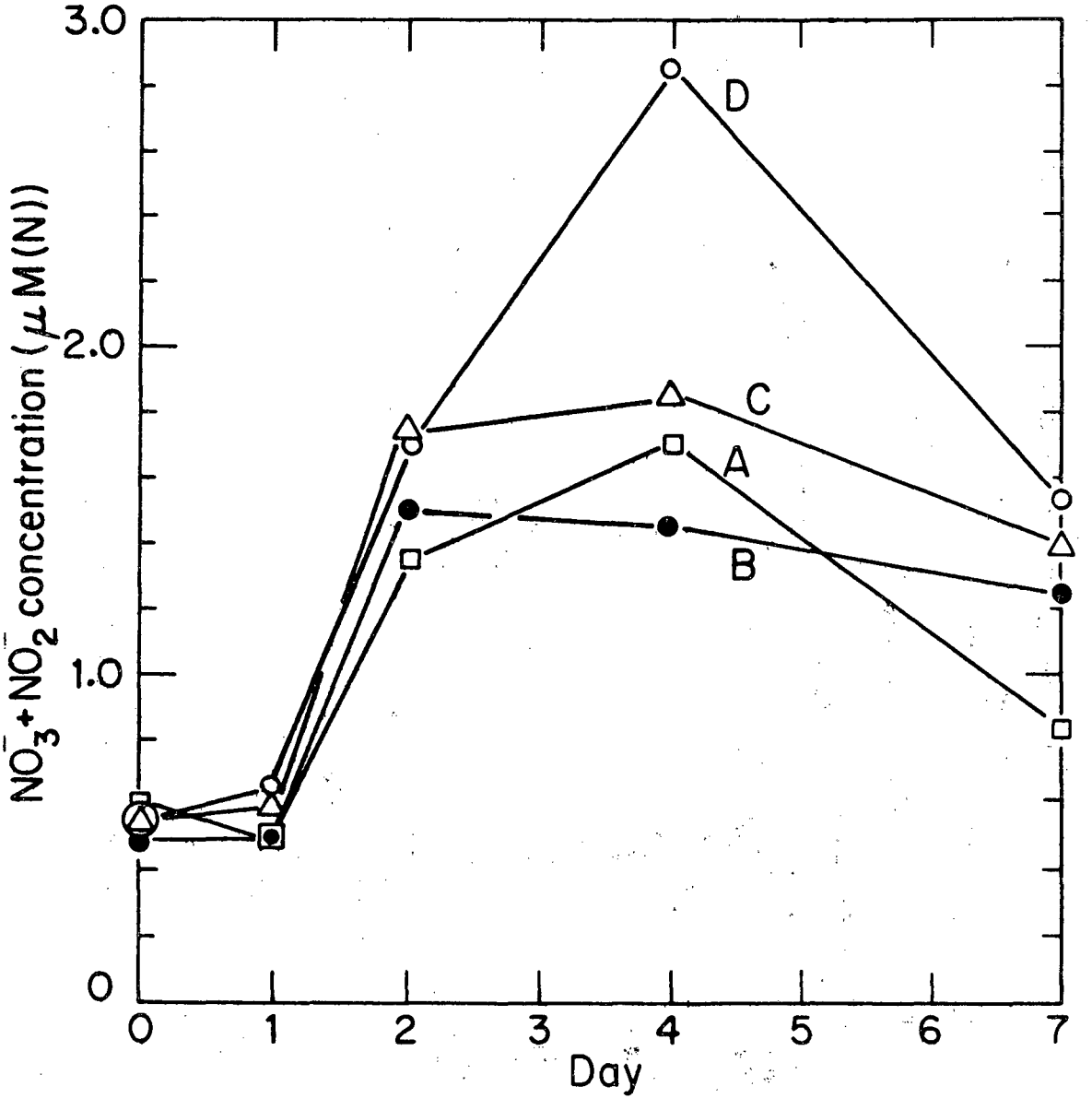

Figure 5-2 NO_2^- + NO_3^- concentrations for control and treatments in K-1.

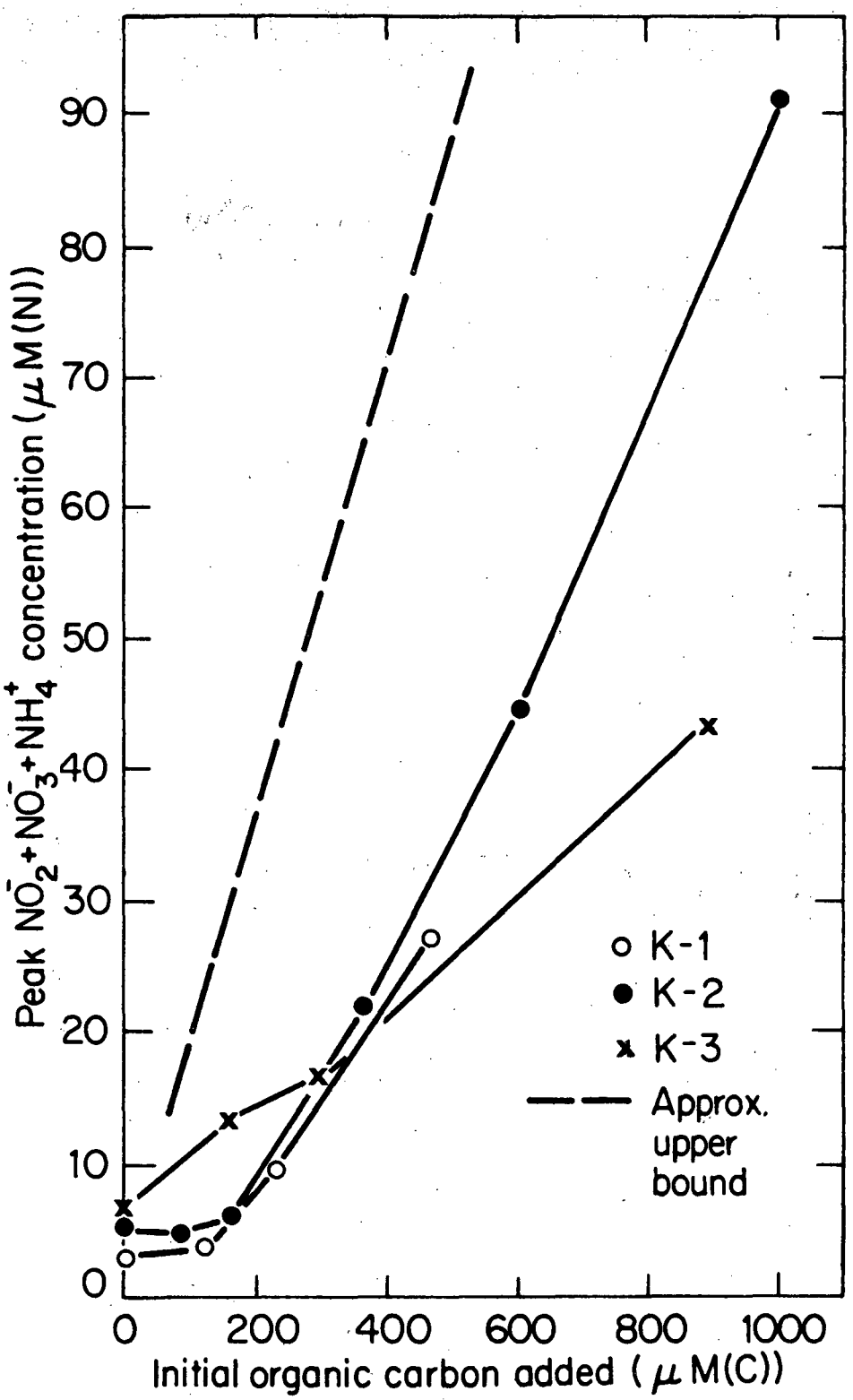

Figure 5-3 The maximum $NH_4^+ + NO_2^- + NO_3^-$ concentration plotted versus the increase in organic carbon for each system in K-1, K-2, and K-3. The dashed line shows the approximate upper bound for the IN concentration assuming that the C/N ratio is 6, that all of the nitrogen present in the added detritus is converted to IN, and that all of the produced IN is present at the time IN concentrations reach their peak value.

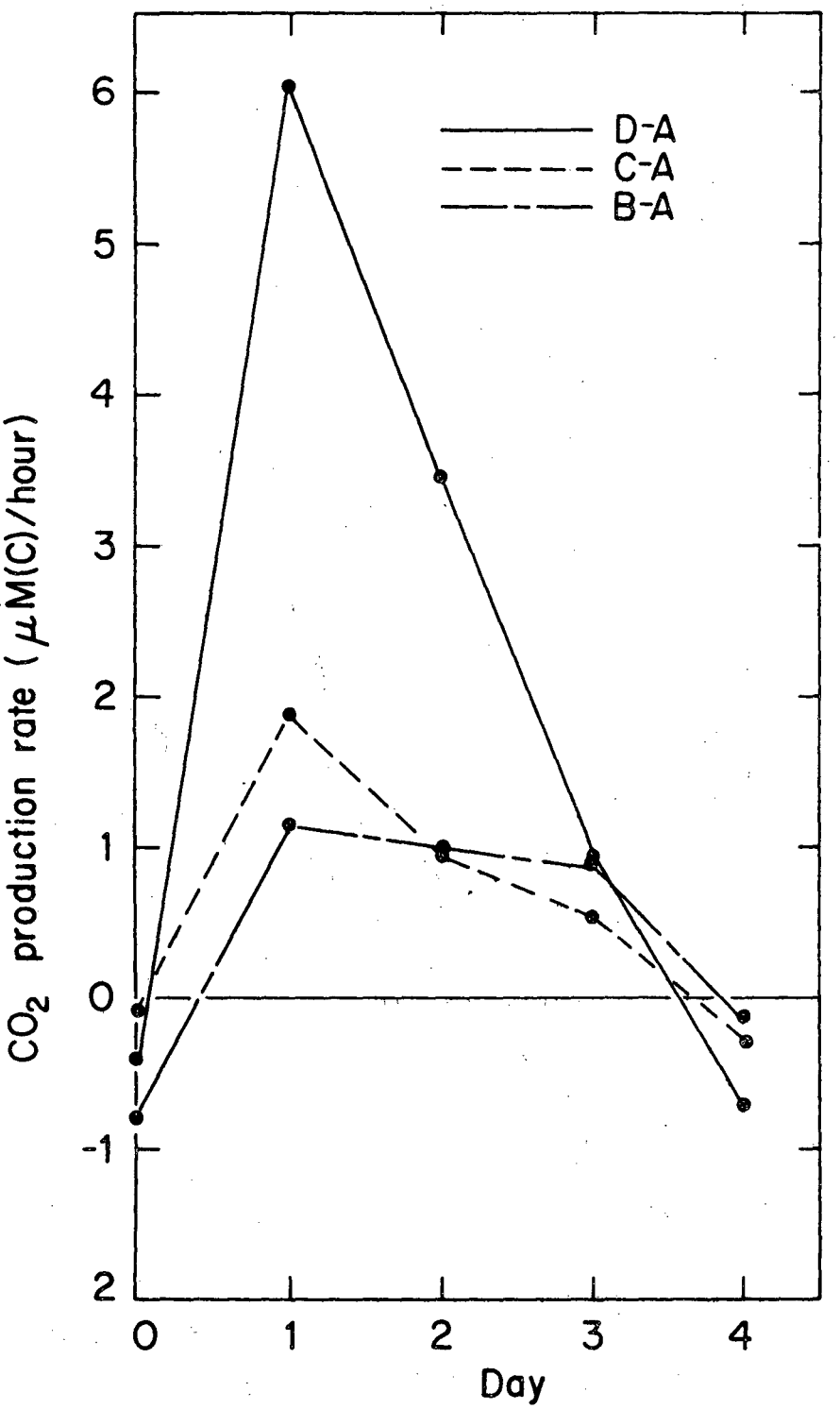

Figure 5-4 The daily dark-bottle CO_2 production rates for K-1. the control value has been substracted from each of the treatment systems' values here in order to display directly the relative effects of te detrital additions. Replicate measurements have been averaged. For reference, the control system measurements for the 5 days of measurement presented here were -2.35, 2.7, 1.3, 2.2 and .75 µM(C)/hour respectively.

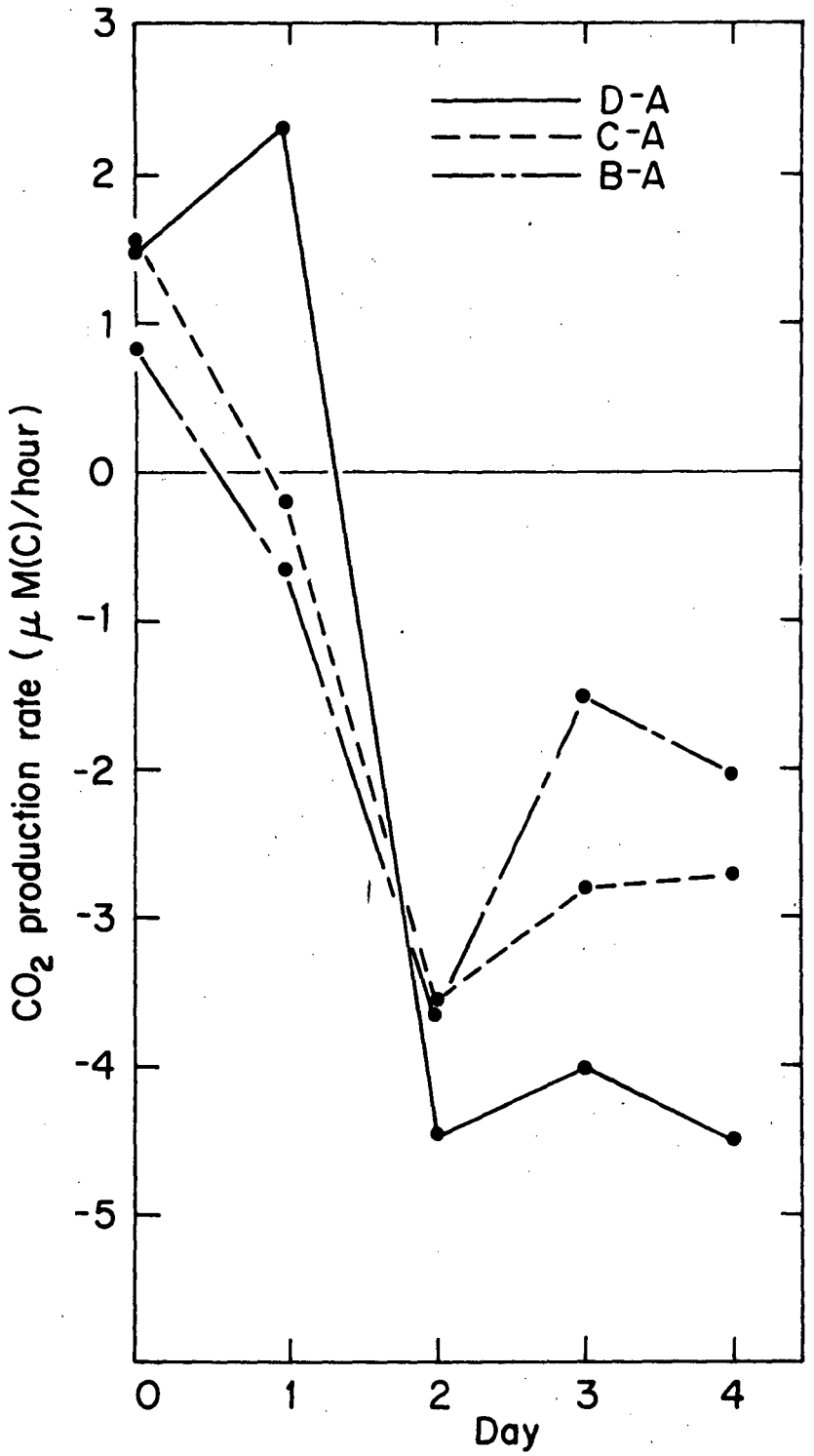

Figure 5-5 The daily light-bottle CO_2 production rates for K-1. The control value has been subtracted from each of the treatment systems' values here in order to display directly the relative effects of the detrital additions. Replicate measurements have been averaged. For reference, the control system measurements for the 5 days of measurement presented here were -4.75, -.15, .1, -.05, and -.65 µM(C)/hour respectively.

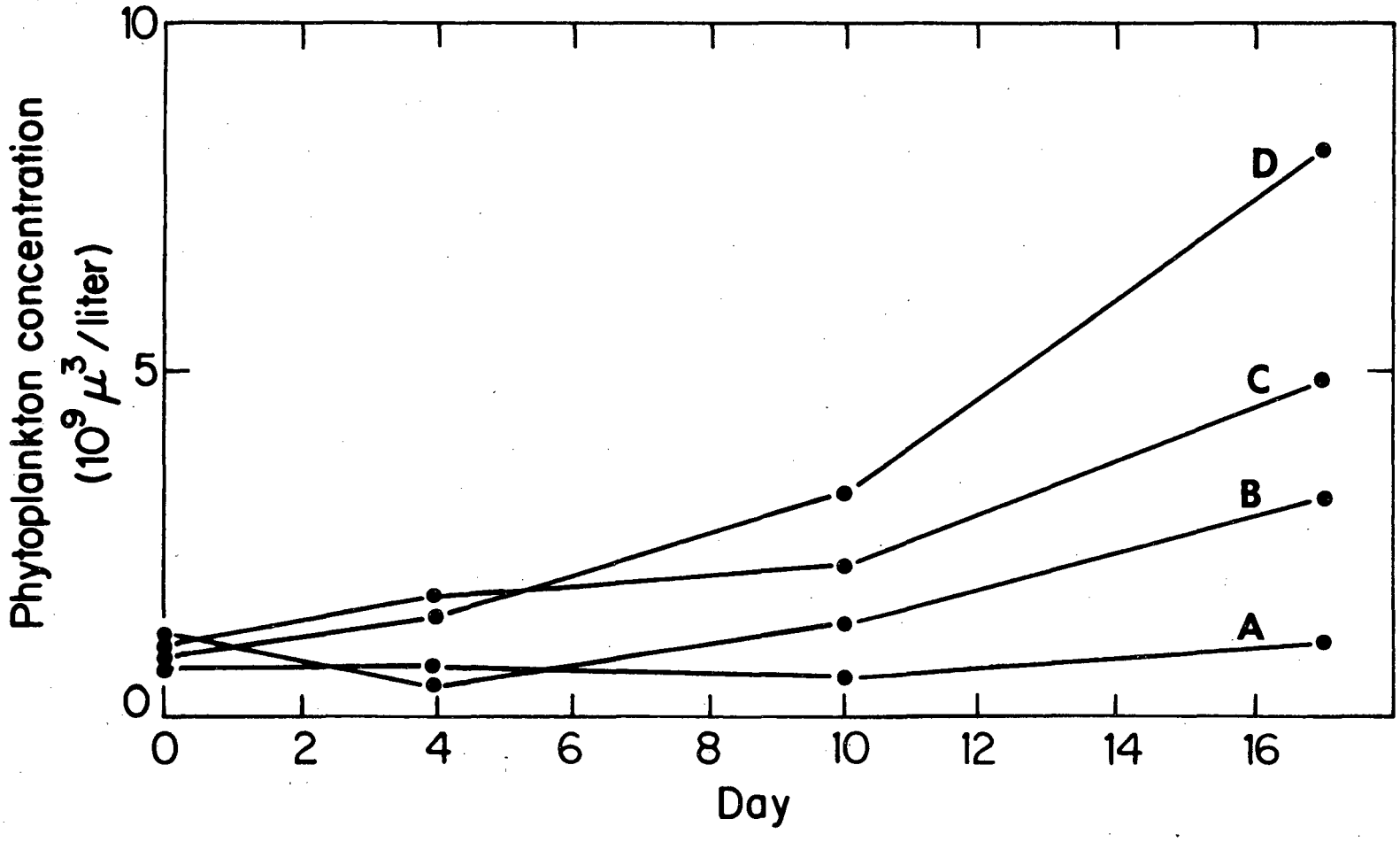

Figure 5-6 Phytoplankton volume densities measured in experiment K-1.

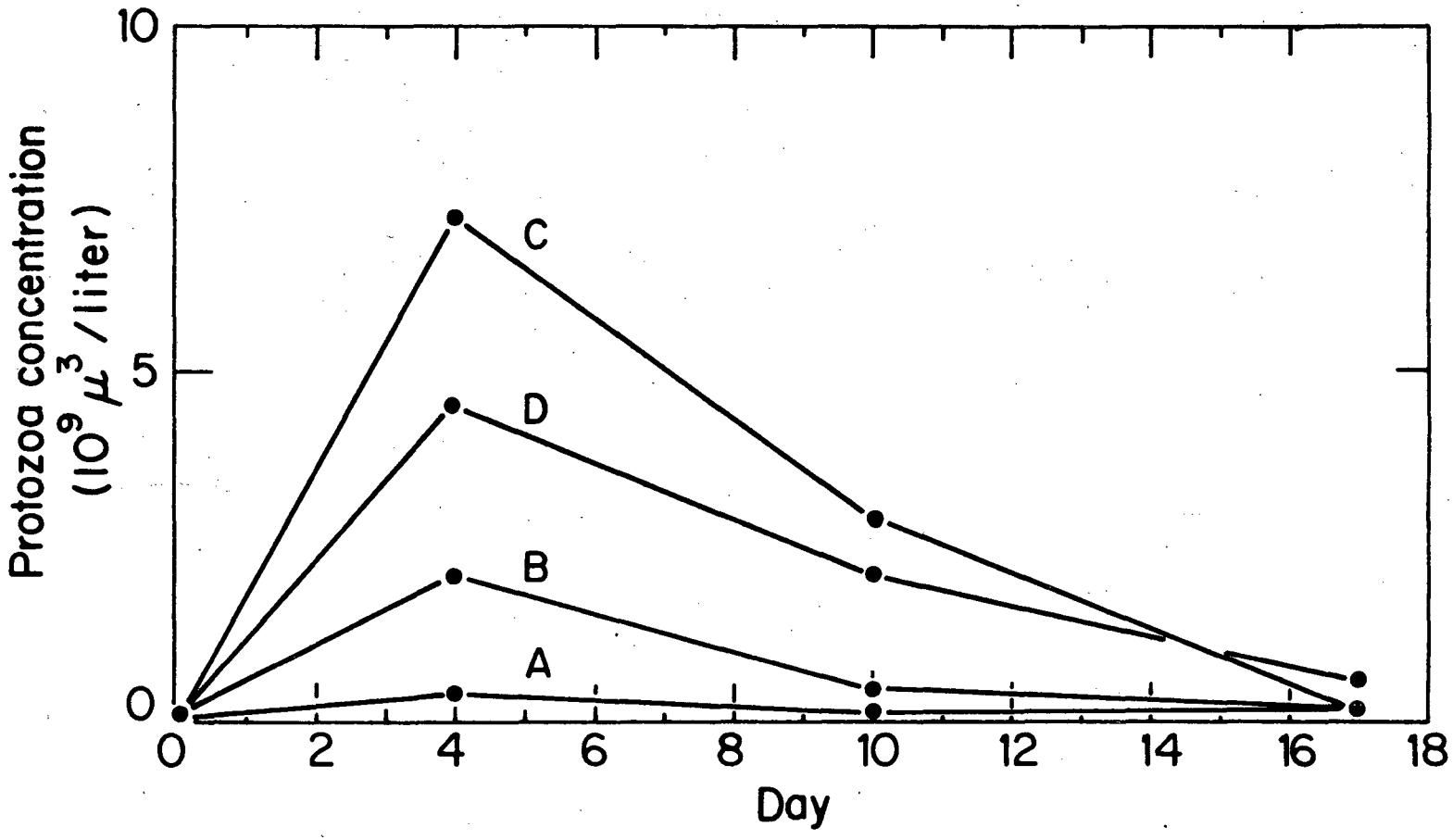

Figure 5-7 Protozoa population volume densities measured in experiment K-1.

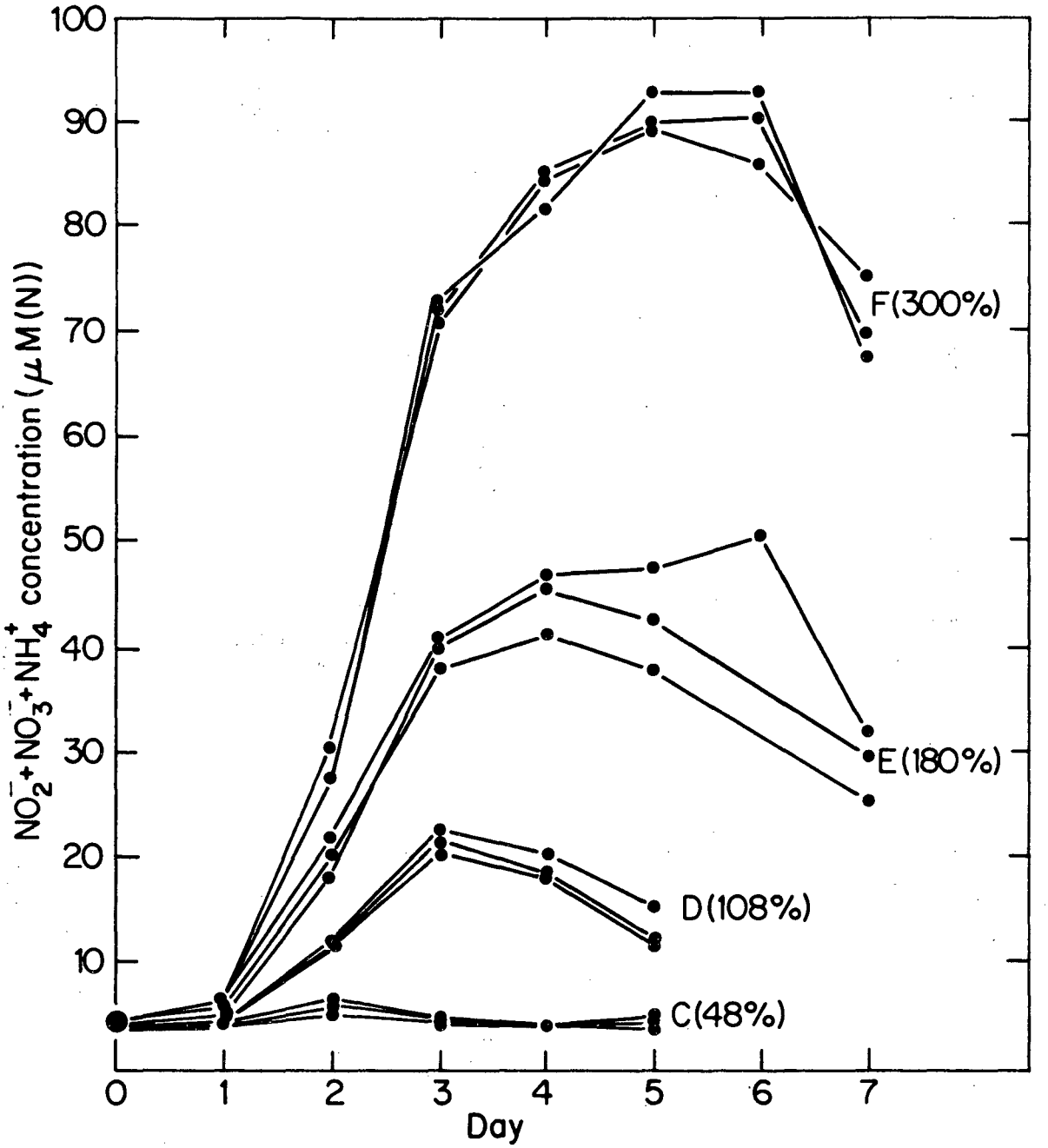

Figure 5-8 NH_4^+ + NO_2^- + NO_3^- concentrations in four of the treatment systems in experiment K-2. Results for systems A and B are not shown; their concentrations were consistently at or below that of system C. Shown in parentheses next to each system label is the percent increase in organic carbon for that system.

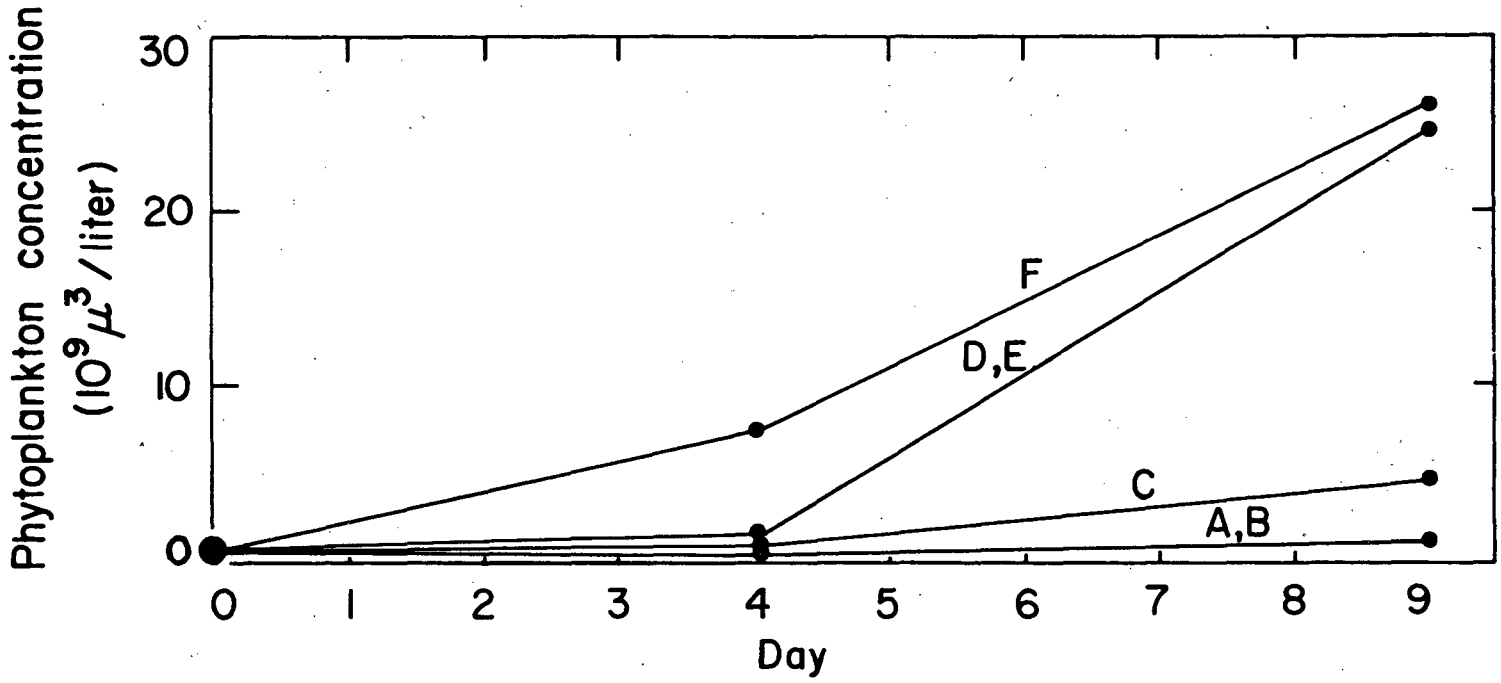

Figure 5-9 Phytoplankton volume densities measured in experiment K-2. Where two systems are represented by a common line, the results for those systems were indistinguishable within estimated measurement error.

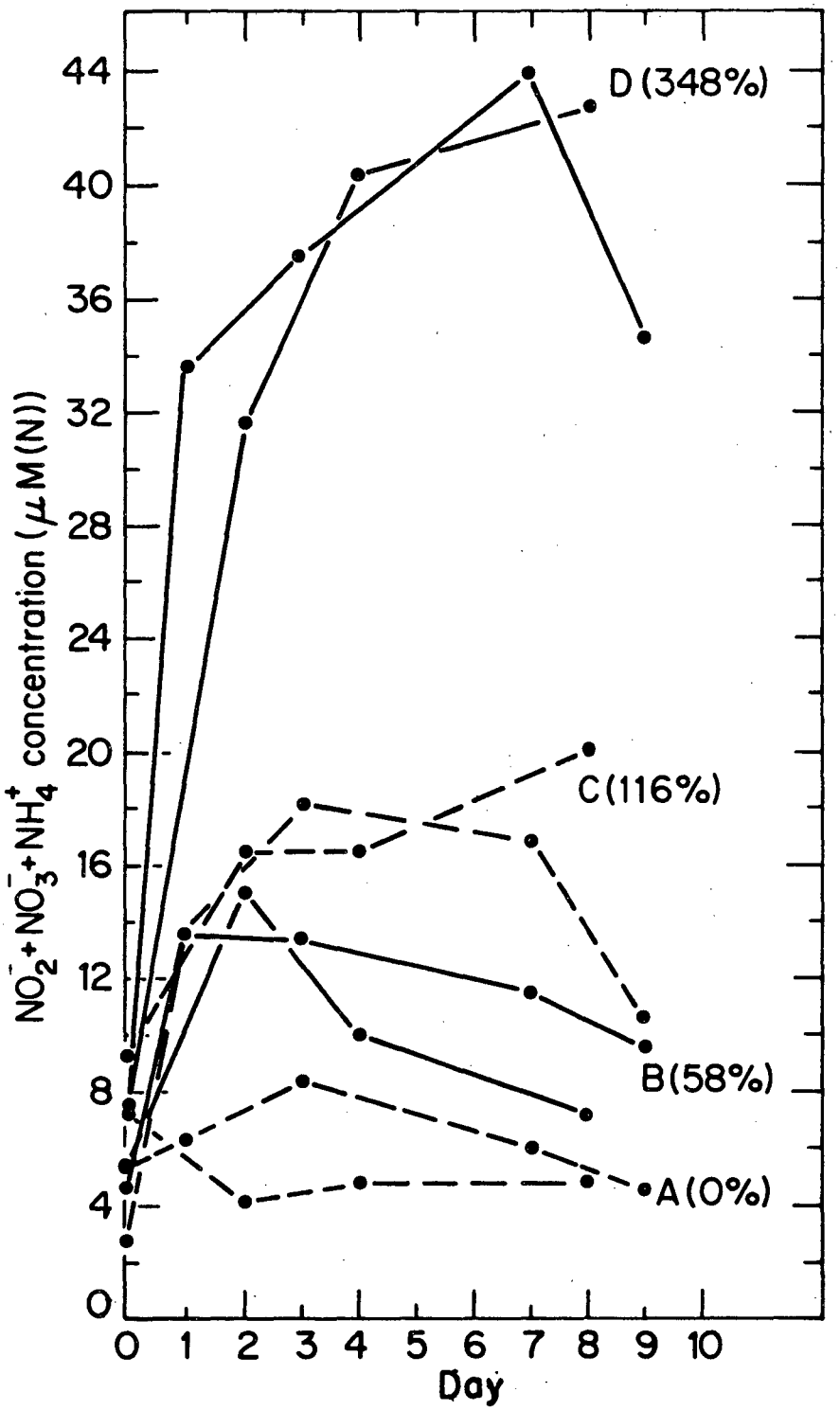

Figure 5-10 $NH_4^+ + NO_2^- + NO_3^-$ concentrations in the treatment (B, C, and D) and the control (A) systems in experiment K-3. Shown in parentheses next to each system label is the percent increase in organic carbon.

Fig. 5-1 shows IN concentrations plotted as functions of time for all the systems. Here, and in the other experiments reported, the substrate was added on day 0 immediately after the day 0 water-column samples for measurement were taken. The IN concentrations in replicate systems for both sets A and B were identical within experimental error and only their average values are shown. Most of the increase in IN was accounted for by NH_4^+, with maximum NO_2^- + NO_3^- concentrations in all systems never exceeding 3 μM(N) (see Fig. 5-2). Measurable induced increases in IN concentrations were only seen in systems C and D, where maximum levels of 9 and 27 μM(N) respectively were measured on day 2. The 3-fold maximum increase in IN in D as compared to C is to be noted, since the amount of detritus added to D was only double that added to C. No significant increases in inorganic nitrogen levels were observed in systems B, even though 117 μM(C) of detritus was added to them. Clearly, in this experiment a threshold value of detritus needed to be exceeded before observable changes in IN concentrations appeared.

Figure 5-3 shows the peak IN concentrations (averaged over replicates) for K-1, 2, and 3, plotted as a function of the amount of organic carbon added in the form of detritus. In K-1 the threshold effect is clearly seen, with peak IN concentration increasing rapidly only beyond a certain initial increase in organic material. The NO_2^- + NO_3^- concentrations and the NH_4^+ concentrations each separately exhibited threshold behavior, as can be seen from Figs. 5-1 and 5-2.

Dark and light bottle CO_2 evolution rates are plotted as a function of time in Figs. 5-4 and 5-5. The control's value has been subtracted from each treatment's values in order to display directly the relative effects of the detrital additions (see figure captions for absolute rates). Through day-3, dark-bottle CO_2 evolution rates in the systems with detritus added (B, C, D) were greater than in the controls (A). The maximum rate in each of the three spiked systems occurred on day 1. The values of these maximum dark bottle CO_2 evolution rates increased faster than linearly with corresponding increases in detritus, which is commensurate with inorganic nitrogen data. Light bottle CO_2 evolution rates showed significant uptake (negative evolution) rates of CO_2 between days 2 and 4 for the spiked systems, with the magnitude of these negative rates ordered as A < B < C < D.

In addition to the CO_2 evolution rate data, the water column phytoplankton data indicate induced primary productivity in the systems with detritus added. Fig. 5-6 shows total phytoplankton volume plotted as a function of time. Between days 5 and 17 the spiked systems showed increases in water column phytoplankton volume with values of 1.2, 3.4, 4.6, and 8.2 x 10^9 μ^3/liter for systems A, B, C, and D respectively being attained on day 17. We caution here that phytoplankton growing on the sides and bottoms of the containers are not included in our counts.

Over the first 10 days subsequent to the detrital additions, total zooplankton volumes in all systems remained low (<.5 x 10^9 μ^3/liter). Between days 10 and 17 they increased in D to 2.5 x 10^9 μ^3/liter, while remaining low (<.5 x 10^9 μ^3/liter) in the other systems. Unlike the zooplankton, protozoa exhibited significant increases over the first 4 days in those systems where detritus was added (see Fig. 5-7). They remained low (<.25 x 10^9 μ^3/liter) in the control systems A. The smallest time interval between successive protozoa measurements was 4 days,, which means that the peak levels might have been missed. Nonetheless, we observed apparent protozoa volume maxima in all spiked systems on day-four with values of 2, 7.3 and 4.5 x 10^9 μ^3/liter obtained for systems B, C, and D respectively.

K-2. Five levels of bacterially derived detritus, corresponding to additions of 82 μM(C), 163 μM(C), 367 μM(C), 612 μM(C), and 1020 μM(C) organic carbon were added to systems B, C, D, E, and F respectively. Fig. 5-8 shows IN levels plotted as functions of time for systems C, D, E, and F. The IN levels in systems A (controls) and systems B (82 μM(C) detritus added) remained constant and low (~1.0 μM(N)) and are not plotted. As in K-1, NO_2 + NO_3 concentrations remained low (<2 μM(N)) in all systems and the IN increases were comprised largely of NH_4^+. The peak IN levels displayed the same threshold effect as in K-1 and indeed the two sets of data as plotted in Fig. 5-3 are nearly overlapping. It should be emphasized that K-1 and K-2 were run nearly 8 months apart and were performed with different sources of lake water. The only major difference between the IN data of K-2 and K-1 is that in the former, with increasing amounts of added detritus, increasing time intervals occurred before the maximum level of produced inorganic nitrogen was attained. A similar phenomenon was observed by Williams and Gray (22).

Figure 5-9 plots total water column phytoplankton volumes as a function of time. By day-9, increases were observed in systems C, D, E, and F, with the total volumes being 4.5, 24.5, 24.5, 26 x 10^9 μ^3/liter) during the experiment.

K-3 and K-4. These two experiments differed from K-1 and K-2 in that the detritus spike consisted of algae rather than E. coli. In K-3, the added detritus contained only dissolved organic matter, while in K-4 the entire algal concentrate, consisting of dissolved plus particular organic mater (DOM + POM), was added. Fig. 5-10 shows IN concentrations as a function of time for K-3, while Fig. 5-3 shows the results of the comparison of the measured peak concentrations of mineralized inorganic nitrogen versus the amount of substrate added. Evidence for a threshold is not observed. Although the replication in K-4 was sufficiently poor that no conclusions about a threshold can be drawn, lower IN concentrations were seen in K-4 compared with K-3. In systems C and D of K-4, for example, the increase in IN was less than half that observed in C and D, respectively, in K-3. This indicates that DOM was more effectively mineralized than was an equivalent concentration of DOM + POM, consistent with the findings of Cole and Likens (25) at considerably lower concentrations of added substrates. In both K-3 and K-4 the fraction of measured IN in the form of NO_2^- + NO_3^- was considerably higher than in K-1 and K-2; in all systems over half the observed IN was NO_2^- + NO_3^-. In K-3 and K-4, as in K-2, the larger the substrate addition, the later in time that the peak IN concentration was reached.

DISCUSSION

Our discussion focuses on the threshold effect seen in the peak IN concentrations in the two experiments K-1 and K-2. In particular, we concentrate on whether this effect actually characterizes net mineralization activity. We recall that net mineralization is mineralization minus immobilization and export. The IN concentrations that we measure do not need to be corrected for immobilization or export losses, prima facie. On the other hand, the measured IN concentrations do not necessarily indicate directly the net mineralization activity in our systems; corrections for uptake of IN by primary producers must be taken into account. It is possible that primary production took place in such a fashion as to produce the threshold effect, even though net mineralization activity was simply proportional to the amount of substrate added. For

example, if uptake in IN for primary production saturated above some critical IN concentration, then the fractional amount removed would decrease with increasing IN concentration, and this could have produced the observed effect.

If the explanation of the effect involves saturation of IN uptake in the process of formation of some product such as algal biomass then a clear signal would be the observation of an amount of product that did not increase as fast as linearly in the amount of detritus added. In contrast, if the amount of product seen also exhibited the threshold effect, then the biological uptake rate most likely reflected, rather than caused, the observed IN threshold phenomenon.

The data shown in Figs. 5-6 and 5-9 lessen the likelihood that nutrient uptake for water-column phytoplankton growth caused the effect. The phytoplankton growth rates and absolute levels in K-1 (Fig. 6-6) suggest that with increasing derital additions, a roughly proportional increase in the water-column phytoplankton production took place. In K-2, the water-column phytoplankton data (Fig. 5-9) even show a threshold effect, in the sense that production in D was considerably greater than that in C, suggesting that phytoplankton growth reflected, rather than caused, the threshold-like large difference between the maximum IN concentration in C and D. On the other hand, the bunching effect observed for phytoplankton growth in D, E, and F suggests that saturation kinetics in inorganic-nitrogen uptake by phytoplankton might have cause the slightly greater-than-proportional increase in peak IN levels as the detritus input increased from D to E to F.

The existence of a threshold in the phytoplankton densities between systems C and D in K-2 does not prove conclusively that there was a threshold in net mineralization activity because herbivory by zooplankton might have become saturated for large phytoplankton densities. However the low zooplankton densities throughout the course of K-2 suggest that herbivory played a minor role in determining phytoplankton densities.

More serious objections can be raised to the argument advanced above, which was based on water-column phytoplankton densities. Most importantly, these measurements do not provide information about uptake of IN by phytoplankton

cells that initially grew in the water column and then subsequently sunk to the bottom of the beakers or attached to its walls. They also do not provide information about uptake and storage of IN in pre-growing phytoplankton cells. Finally, they do not provide information about uptake of IN by algae attached to the surfaces of the beakers.

To address these objections, we turn to the dark- and light-bottle CO_2 evolution measurements of K-1, shown in Figs. 5-4 and 5-5. From these data an argument can be advanced to suggest that the threshold phenomenon characterized the mineralization process, itself, and was not an artifact of the uptake kinetics of IN by phytoplankton.

We write:

$$L = P + Q + R$$

$$D = Q + R$$

where L and D are the light- and dark-bottle CO_2 production rates, respectively, Q is the contribution to CO_2 production from gross mineralization (including zooplankton respiration) minus immobilization, P is the gross primary production contribution to CO_2 production in the light, and R is the phytoplankton respiration contribution to CO_2 production. Note that P will often be negative with our sign convention. It is then straightforward to show that on day-1, when CO_2 production was maximum, the Q's are a faster-than-linearly increasing function of added detritus for any fixed P/R ratio satisfying $0 \leq R \leq -P$. This is illustrated in Table 5-7, which gives the value of Q on day-1 for 3 different assumed values of P/R. We cannot estimate reliably from the closed-bottle data what the net amount of algal growth actually was, as that quantity is very sensitive to the value of P/R.

Closed-bottle NH_4^+ production measurements in K-1 provide further evidence against the possibility that the kinetics of uptake of ammonia by algae on the surfaces of the 4-liter vessels was a significant cause of the threshold effect. The closed bottles could not have developed appreciable surface growth over the 4-hour period of measurement. Nevertheless, these closed-bottle NH_4^+ production rates clearly exhibited the threshold effect and were

Table 5-3

VALUES OF Q (THE CONTRIBUTION OF MINERALIZATION TO THE RATE OF CO_2 EVOLUTION) ON DAY-1 of K-1, FOR 3 ASSUMED VALUES OF THE RATIO OF P TO R (SEE TEXT)

	R = 0	R = -P/2	R = -P
$Q_D - Q_A$	6.05	4.20	2.35
$Q_C - Q_A$	1.90	0.90	-0.15
$Q_B - Q_A$	1.15	0.25	-0.65
Q_A	2.70	1.30	-0.15

consistent with the results from our measurement of daily water-column NH_4^+ concentrations. We note that these measurements were made in water-column samples that did not include any of the added detritus which may have sunk to the bottom of the 4-liter containers. The fact that the threshold effect was seen suggests that it reflected water-column activity and was not due to the proportionally greater amount of detritus which may have settled to the bottom of D or C as compared with B.

Uptake of IN by denitrifying bacteria can also be considered as a possible cause of the loss of significant quantities of NO_2^- + NO_3^- from the water column of our beakers. A saturation phenomenon in the kinetics of this process, could have generated the observed threshold effect. As discussed in the introduction, however, denitrification of mineralized IN is not a correction that need be applied to the IN measurements in order to obtain the net mineralization rate. In addition, the O_2 saturation maintained in our beakers makes it unlikely that denitrification could have depleted a large fraction of the produced NO_3^- + NO_2^-.

One other possible explanation of the threshold phenomenon deserves mention. Some NH_4^+ is known to adsorb onto the surfaces of particles (27), and this fraction of the produced NH_4^+ would escape detection by our measurement procedures. If particle-surface-area were adequate to adsorb a relatively large fraction of the NH_4^+ produced in systems B, but not in the systems with larger amounts of added detritus, then a threshold effect would appear. The difficulty with this explanation is that the amount of particle-surface-area added to each of the systems in K-1 and K-2 was proportional to the amount of organic carbon added, and therefore such a saturation effect is unlikely.

If, as we have argued, the threshold effect characterizes net mineralization activity, it is then pertinent to inquire as to the mechanism responsible for this effect. The measurements of protozoa population density in K-1, shown in Fig. 5-7, provide some information in this regard. Predation on bacteria by protozoa has been widely reported (20,29). Our observed increases in protozoa population densities very likely reflect increases in bacteria population densities. Fig. 5-7 shows that the maximum protozoa density in system D, was actually below that of C. This reduces the likelihood that in system D, with twice as much substrate added as in system C, the bacteria population grew

twice as large as in system C. Saturation of bacterial biomass growth (as a function of increasing substrate) can be inferred, and as discussed in the introduction, this could have generated the threshold in net mineralization activity.

CONCLUSIONS

Our original hypothesis was confirmed in two of the four experiments (K-1 and K-2) designed to test it. In K-4, poor replication did not allow a test. In K-1 and K-2, utilizing detritus of bacterial origin, a threshold effect was observed, while in K-3, involving detritus of algal origin, no threshold effect was observed. The analysis of all the data from K-1 and K-2, particularly the IN concentrations, the sealed-bottle measurements of CO_2 changes, and the protozoa densities, suggests strongly that the observed threshold phenomenon characterizes detritus-decomposer interactions and is not simply a reflection of the kinetics of uptake of inorganic nutrients by phytoplankton. A qualitative picture utilizing a simple microbial carrying capacity mechanism is one possible way of viewing the data. Above a certain population density, in this model, decomposer growth (immobilization) ceases while mineralization continues at significant rates. From this point of view, we would say that the initial conditions (water source and type of detritus) of experiments K-1 and K-2 allowed this carrying capacity to be reached within the range of detritus additions. Within this framework, we can deduce that a threshold in net mineralization in K-3 was missed because that threshold corresponded to a level of added substrate either below the lowest level added or above the largest level. The rapid onset of net IN production seen in K-3 (Fig. 5-10) suggests that the threshold was below the lowest level added.

The increasing delay in the time at which peak IN concentrations were seen in K-2, K-3 and K-4 as the detritus addition increased is consistent with the observation of Williams and Gray (22) of increasing induction periods (see introduction). This behavior can be simulated by Michaelis-Menten kinetics for the uptake of detritus by decomposers, but that same model analysis gives rise to curves in Fig. 5-3 that bend over with a nonlinearity opposite to that seen in K-1 and K-2.

From studies of ecosystem stability, the importance of being able to measure density-dependent saturation effects in populations can be inferred (30).

However, the empirical accessibility of the concept of density dependence has been a subject of controversy (31-33). Empirical evidence of density-dependent regulation in populations of decomposers is conflicting, at best. Hairston et al. (34) argued that decomposers are generally food-limited in nature, while Potter (35) concluded that in aquatic systems the number of benthic bacteria present limit the rate of decomposition (presumably because factors other than food limit their numbers and activity). Much of the discussion on this topic has taken place within the context of attempts to search for and quantify density dependence of correlation analysis, in which the changes in a population over a sequence of fixed time periods are examined to see whether the changes depend nonlinearly upon the population. As shown by Eberhardt (36), this approach is beset with statistical traps. It is suggested here that appropriately-chosen detritus manipulations followed by measurements of mineralization products, offer a means of identifying and quantifying microbial carrying capacities in aquatic systems.

Section 6

CONCLUSIONS

The results of our pelagic microcosm assessment studies point to the following conclusions:

- Good replication can be achieved for at least 56 days, regardless of the particular microcosm configuration, if care is taken during initiation procedures.
- With surface-growth eliminated, there is a range of sizes (50-150 liters in our case), where behavior of microcosms of similar depth does not depend on size.
- Good tracking can be achieved with 50- and 150-liter poured systems for 56 days, when seasonal and other natural conditions are such that temperature differences between microcosms and the natural system are small, and when there is little import of nutrients to the lake epilimnion. Our microcosms, which were designed to model the isolated lake epilimnion, behaved most like their parent lake during the season when the surface waters of the lake were indeed most isolated and physical conditions were most similar to those in the laboratory.

It is clear from our results that pelagic microcosms must be used with caution in toxic substance assessment. For certain purposes, they appear to be appropriate, while for others, inappropriate. The longer they are run, the greater the divergence between their behavior and that of their parent water body; thus their use for short-term testing is more appropriate than for long-term testing. During certain seasons, when the water columns of lakes are subject to physical conditions rendering them particularly isolated from their sediments and the surrounding watershed, the time period over which microcosms behave realistically is increased.

Tests of the effects of toxic substances on system-level nutrient regeneration properties will yield more realistic results than tests of effects on planktonic succession patterns. Because the former type of test can, in principle,

be carried out in a relatively shorter time period than the latter, due to the rapidity with which microorganisms respond to food sources, it is particularly appropriate for microcosm applications. The generally satisfactory replication and tracking of nutrients reinforces this conclusion. Further motivation for pursuing application of aquatic microcosms to assess toxic substance effects on nutrient-microorganism-detritus processes is provided by the results of experiment VI, which demonstrated that microcosms can provide replicable and useful information about these processes. Such information could not be obtained in any practical fashion from field investigations.

The cautionary comments made above concerning long-term applications of pelagic microcosms are based on the state-of-the-art evaluations reported here. At the same time that this work has delineated present limits to effective microcosm applications, it has also pointed to possible ways of improving pelagic microcosms so as to increase their range of applicability. This is discussed in the following subsection.

IMPROVEMENTS IN PELAGIC MICROCOSM TECHNOLOGY

The goal of the improvements suggested here is to make microcosms more like their parent natural system for longer time periods and to make them easier to operate. They involve modifications of procedures at the natural water body as well as within the laboratory. They can be arranged according to monitoring procedures, initiation procedures, and laboratory conditions.

Monitoring Procedures

1) At the natural water body, increasing the number of time-depth profiles made of the pertinent variables will aid greatly in understanding the degree of microcosm tracking as well as suggest ways to optimally initiate the microcosms. In particular, it should illuminate various plankton vertical migration or location patterns.

2) Some measure of the degree of water agitation in the natural system should be carried out, perhaps using gypsum dissolution. If warranted by these measurements, paddle agitation systems should be installed in microcosms.

3) Additional variables should be measured. These include O_2, pH, primary productivity, and silica.

Initiation Procedures

1) Depending on initial depth profiles measured in the natural systems, an integrated water column sample should be used to initiate microcosms. This should include enough of the water column to obtain a good representative taxonomic and chemical sample. In particular, we hope to eliminate, at least partially, the discrepancy seen in large cladocera and copepod numbers between the microcosms and the natural system. Also, we hope the lower light levels in microcosms are more appropriate as a representation of the light levels of the vertically integrated water column.

Laboratory Conditions

1) As previously mentioned, paddle agitation systems should be installed if the natural water body experiences significant agitation levels.

2) Attempts to match the natural system's temperature in the microcosms should be made. In most cases, this usually means cooling the microcosm systems.

3) An attempt to demonstrate good tracking with systems smaller than 50 liters, perhaps 15 liters, should be made. Small systems have the advantages that they are easier to initiate because they need less water, and they are easier to maintain at a given temperature than larger systems. Two possible configurations which might work are suggested by our results. First using small-volume, narrow cylinders whose height is about the same as the 50 liter cylinders is one approach suggested by the size independence of poured 50 and 150 liter systems which were of the same height. Second, using siphoning as opposed to pouring to eliminate surface growth might reduce the small system NO_3^- + NO_2^- increases observed in poured systems. This possibility is suggested by results seen in experiment I.

We recall that in experiment III, the 15-liter poured systems exhibited good phytoplankton tracking but increased levels of NO_3^- + NO_2^- compared to the natural systems. To explore the possibility that good tracking without the NO_3^- + NO_2^- increases could be achieved in 15 liter systems we suggest one possible experiment. Three sets of replicate 15-liter microcosms would be

initiated from a natural water body. They would be subjected to the following water transfer methods to eliminate surface growth: (1) pouring all contents to clean containers (2) siphoning all contents to clean containers, and (3) siphoning all contents but the bottom 1 cm to clean containers. This simple experiment would allow comparison of effects induced in having different amounts of bottom materials left in the systems and comparison of different agitation rates (pouring versus siphoning).

Finally, we discuss below ways of extending the range of usefulness of lake microcosms that go beyond improving their ability to simulate the pelagic zones of lakes.

EXTENSION OF THE RANGE OF APPLICABILITY OF LAKE MICROCOSMS

The most immediate and useful extension of our systems would be to include a sediment compartment. This would allow for testing of toxic substances whose effects and partitioning are significantly mediated by sediments. To this end, we are designing sediment packages to be included in our microcosms. Once included, tracking experiments similar to our pelagic epilimnion tracking experiments will be carried out to test the realism and replicability of microcosm models of shallow lakes and the hyplomnion zone of deep lakes.

While it would be highly desirable to use microcosms to study effects of toxicants on fish, unfortunately the presence of fish greatly distorts the behavior of small ($\leq$1 m^3) microcosms (37). Other effects that cannot be studied directly in microcosms include changes in water clarity or odor build-up over long periods of time. Often the quantities that cannot be modeled realistically in microcosms are the ones of direct concern to society. We call such variables "macrovariables." A careful analysis of this problem by Gleick (38) has pointed the way in which microcosms can still be of great use in this connection. The basis of the approach is to combine field observations with microcosms studies. Field investigations have revealed a number of linkages between the microvariables (variables that potentially can be tracked adequately in a microcosm, such as the nutrient concentrations, plankton densities and mineralization rates) and the macrovariables that are of direct concern to society, such as sports-fish production rates. Thus, if microcosms are used to study effects of toxicants on the microvariables, then deductions can be drawn concerning effects on the macrovariables.

To study direct effects of toxicants on fish, however, the best approach probably will remain single-species test procedures, despite their problems. One means of making such single-species tests more realistic is to insure that the toxicant tested has undergone realistic transformations within the test system. Many of these transformations are effected by the chemicals and microorganisms present naturally in lake water. Microcosms can be of use in providing a realistic conditioning environment for toxicants. A substance to be tested on fish might first be placed in a lake microcosm for a prescribed period; subsequently water would be transferred from the microcosms to the fish tanks where effects on the fish would be noted.

It is of considerable importance to match microcosm uses to microcosm characteristics. A number of toxicants, for example, could not be tested realistically in microcosms. Several common-sense criteria immediately suggest themselves:

1) If the substance is to be tested in a pelagic microcosm, then in natural systems it must not have major pathways to the benthos or undergo significant transformations in the benthic zone.

2) The major effects of the substance must not be on macrofauna or macroflora, as these components will be absent from the microcosms.

3) The substance must not rapidly leave the water column and absorb on the side of the container.

4) The substance must not volatilize at a different rate in the microcosms than it would in the natural system.

5) The substance should not be too UV sensitive, as matching external UV intensities is difficult in the laboratory.

Pretesting of toxicants in microcosms to determine pathways, volatization rates, and degradation rates should precede actual effects testing where such information is not already available or deducible. As in effects testing, the replicability and realism of microcosms used for pretesting should be evaluated carefully. It may not be necessary in all cases to impose as stringent requirements on the pretesting systems as on the effects testing systems,

although this will have to be decided on a case-by-case basis. Consider the case in which the phytoplankton succession pattern in the microcosm differs from that in the field, but overall community respiration rates are similar. Then such microcosms may yield sufficiently reliable information about metal methylation pathways even if the microcosm results about effects of the methylated products on the plankton succession patterns are misleading. On the other hand, an aquatic microcosm may give reliable information about the effects of a toxicant on mineralization rates but be of little use in identifying and characterizing a biomagnification pathway because of the exclusion of macrofauna.

To conclude, pelagic microcosms have a role to play in ecotoxicology, but they must be used with care. Adequate replication and realism are achievable, but only under limiting conditions described here. These conditions impose constraints on the types of applications as well as on microcosm design and operation. The goal of cheap, realistic, replicable test systems for ecotoxicology is an appropriate and sensible one toward which industry and scientists can move. It is unlikely, however, that any one such system will be of widely generalizable use. For the future, the most important task in this regard is to develop test protocols for existing, proven systems and to expand the range of available test systems, delineating clearly the constraints on their use.

Section 7

APPENDIX A: METHODS OF DATA ANALYSIS

DEFINITIONS

For each measured variable, z_i, or combination of measured variables, $f(z_1, \ldots, z_n)$, there is a probability density, $\rho_i(z_i)$ or $\rho_f(z_1, \ldots, z_n)$, associated with that experimental quantity or combination of experimental quantities. The probability density, $\rho_f(z_1, \ldots, z_n)$, for a combination of experimental numbers can be derived from the probability densities for each of the experimental numbers, $\rho_i(z_i)$, and the explicit function, f. From a probability density, various expectation values of the variables are calculated, and these expectation values allow us to assign errors or degrees of accuracy to each experimental number, z_i, or combination of experimental numbers, $f(z_1, \ldots, z_n)$.

The expectation value of the variable z_i, $E[z_i]$, is the mean value (α_i) of the probability density associated with z_i; the expectation value of the variable minus the mean value squared, $E[(z_i - \alpha_i)^2]$ is the variance, σ_i^2, of the probability density associated with z_i. The positive square root of the variance is the standard deviation, σ_i. These three quantities, α_i, σ_i^2, and σ_i can be referred to as the mean value, variance, and standard deviation associated with z_i. A parallel set of definitions holds for a combination of variables, where we consider the combination of z_i's as a single variable, $y = f(z_i, \ldots, z_n)$.

The mean value of a probability density is sometimes referred to as the true or underlying mean value of the parameter measured, because it is usually what the measurement is trying to determine (39). For simple probability densities, the standard deviation of the probability density gives a good estimate of the error associated with each experimental number or combination of numbers. For example, for a gaussian probability density, the 90% confidence interval for determination of the true mean, α_i, of the probability density by the measurement, z_i, is

$$z_i - 1.65\ \sigma_i \leq \alpha_i \leq z_i + 1.65\ \sigma_i \qquad \text{(A-1)}$$

or $\alpha_i = z_i \pm 1.65\ \sigma_i$ with a 90% degree of confidence.

For other probability densities, a given confidence interval or error about the true mean value may depend on other quantities in addition to the variance of the probability density. Moreover, the standard deviation or variance of a probability density, may depend on the mean value of the probability density, α_i, as well as other quantities. For each experimental variable, the appropriate probability density as well as functional dependence of its variance is determined by the interaction of measurement techniques used and the intrinsic properties of the system measured.

PROBABILITY DENSITIES AND STANDARD DEVIATIONS ASSOCIATED WITH INDIVIDUAL MEASUREMENTS

A series of calibration experiments was carried out in order to determine probability densities and standard deviations associated with these probability densities for three important variables: NO_3^- + NO_2^-, NH_4^+, and phytoplankton volume densities. We assumed that the probability densities and their associated standard deviations for each particular measurement were the same in the general experiments as in the calibration experiments. In addition to estimating the error associated with a single measurement of the above experimental quantities, we used the assigned probability densities and standard deviations to determine the errors associated with various combinations of experimental parameters used to assess replication and tracking.

The calibration experiments to determine the precision of nutrient measurements were carried out in lake water and in tanks of distilled water to which various amounts of NH_4^+ and NO_2^- + NO_3^- were added. For nutrients no spatial heterogeneity was observed in the tanks, and it was assumed that a gaussian probability density was sufficient to describe z_i, the individual measurement in any tank. The calibration measurements estimated how the standard deviation, σ_i, associated with each probability density depended on a power of the true mean value, α_i. Table A-1 summarizes our results for both nutrients in the range 1 μM(N) $\leq z_i \leq$ 20 μM(N), based on 10 to 20 measurements performed at each of 5 different predetermined concentrations of each nutrient.

Table A-1

STANDARD DEVIATIONS ASSOCIATED WITH NUTRIENT MEASUREMENTS

Nutrient	$\sigma_i = a(\alpha_i)^b (\mu,(N))^{1-b}$	r
$NO_3^- + NO_2^-$	$\sigma_i = 0.3287 (\alpha_i)^{0.717} (\mu M(N))0.283$	0.99925
NH_4^+	$\sigma_i = 0.2086 (\alpha_i)^{0.5} (\mu M(N))0.5$	0.99998

To determine a numerical value for the standard deviation, σ_i, associated with any one particular measurement, z_i, we substitute z_i for α_i in the above expressions.

Counts of non-attached or unclumped bundles of phytoplankton were consistent with a poisson probability density function describing their numbers (40). In other words, these phytoplankton were randomly distributed in the tanks and sampling chambers. We calculated phytoplankton volume densities, by multiplying the number of cells per unit volume by a standard cell volume. The systematic error which is introduced by error, if any, in this standard cell volume has very little impact on our results and conclusions. This is because we almost always compared volume densities within the same size class with one another, so that the standard cell volume is just an over all multiplicative factor.

For a small sample volume, v_s, there is a true mean value, η_i, for the number of phytoplankton cells to be in that random sample volume. If the standard phytoplankton cell volume is v_o, then the true mean value of the phytoplankton volume density, α_i, is $\eta_i v_o / v_s$. Assuming that a poisson density describes the phytoplankton cell numbers in our tanks, the standard deviation for the phytoplankton volume density measurement is

$$\sigma_i = \sqrt{\eta_i}\, v_o / v_s$$
$$= \alpha_i / \sqrt{\eta_i} \qquad \text{(A-2)}$$

To determine a numerical value for the standard deviation associated with a particular phytoplankton volume density measurement, z_i, we substitute z_i for α_i and the actual number of counts in the sample chamber, n_i, for n_i. For most of our measurements the number of cells counted, n_i, usually ranged between 50 and 100.

For clumped phytoplankton, repeated counts were used to estimate the accuracy of a single measurement of such phytoplankton in a tank. In this situation a conservative estimate for a 90 confidence interval for the true mean value, α_i, can be taken as

$$z_i - 0.75\ z_i \leq \alpha_i \leq z_i + .75\ z_i \qquad \text{(A-3)}$$

where z_i is one measured value.

Spatial heterogeneity and often relatively low numbers of zooplankton rendered precise quantitative use of much of the zooplankton data unwarranted. In those few cases where statistically significant results can be quoted, we will simply exhibit the associated variance or standard deviation.

We studied two lakes in our tracking experiments. In those instances when replicate measurements were made in the same small volume of lake water and compared with the same number of measurements made in one tank, the variances measured in the two types of systems were consistent with one another. Thus the most reasonable approach was to assign the same precision to individual measurements made in the lake as in the tanks. This does not preclude the possibility that a variable in the lake will have different values at different depths or horizontal positions.

Typical statistics distributions, much used to analyze biological data, such as the t and chi-square distributions, require that the standard deviations associated with each measurement, although unspecifical a priori, be identical for each measurement (41). Further, as most commonly applied (41), they require replicate measurements which have the same true mean values (α_i). Contrary to these assumptions (as experimentally demonstrated), in our putative replicate systems the true mean values of a given parameter very likely differ among the replicates. Similarly, the standard deviations

associated with these true mean values differ among the replicate systems. Hence we were required to develop our own statistical techniques for assessing replicability and tracking. Our knowledge of the individual standard deviations for individual measurements allows us to estimate accurately the errors associated with the combinations of experimental numbers we used to describe replication and tracking.

AGGREGATION OF DATA OVER TIME

There are two reasons to aggregate or integrate data over time. First the jitter inherent in natural systems' parameters is smoothed out to some degree by this process. Secondly, it allows for sensible comparison between different systems which may differ from one another in one or more of their variables only by a phase difference in time. For example, two freshwater systems may both exhibit a similar diatom bloom but be slightly out of phase if the bloom in one precedes that in the other by a few days. The time-aggregated value for diatom volumes would be similar for both systems, whereas a day-by-day comparison of the diatom populations would yield different results. For our microcosms and lakes such phase differences were judged not to be biologically significant, and so we used time-aggregated quantities in our evaluation of replication and tracking.

Consider a given time interval which includes N data points equally spaced in time in the i'th replicate (z_{ia}, a = 1,...,N). The time-aggregated quantity, x_i, is defined by

$$x_i = \sum_{a=1}^{N} k_a z_{ia} \qquad \text{(A-4)}$$

where

$$k_a = \frac{0.5}{N-1} \quad , \quad a = 1 \ , \ N$$

$$k_a = \frac{1}{N-1} \quad , \quad a = 2,\ldots,N-1 \qquad \text{(A-5)}$$

The true mean value, α_i, and the standard deviation, σ_i, associated with aggregated quantity x_i are

$$\alpha_i = \sum_{a=1}^{N} k_a \alpha_{ia}$$

$$\sigma_i = \sqrt{\sum_{a=1}^{N} k_a^2 \sigma_{ia}^2} \quad , \qquad \text{(A-6)}$$

where α_{ia} and σ_{ia}^2 are the true mean value and variance associated which each data point z_{ia}. The expression for σ_i is strictly true, if the x_i have Guassian probability densities and is approximately satisfied for other probability densities.

VARIABLES USED FOR REPLICATION AND TRACKING

The qualitative analysis in Section 3 was based primarily on time-aggregated quantities. The statistical analysis described below is applicable to any time series, be it time-aggregated or day-to-day. For a given measurement, x_i, in the i'th replicate there is a true mean value, α_i, and a variance, σ_i^2, associated with this measurement. Similarly, associated with the same type of measurement, x_L, made in the lake there is true mean value, α_L, and a variance, σ_L^2. In our analysis, we do not assume that the putative replicates have the same true mean. We define below the combinations of experimental numbers used to discuss the varying degrees of replication and tracking seen in our experiments. The true mean values and variances associated with each of these combination of experimental numbers are given below and in accompanying tables.

Our replicate systems were n separate tanks which, to the best of our ability, were initiated similarily and then maintained under similar conditions. Because each tank contained a complex assemblage of interacting biota and chemicals, we did not assume that the n putative replicates were identical. Rather we treated them like n individual systems and determined within the accuracy of our measurements how close or far from each other their true mean values, α_i, were. To begin, we define the experimental mean among replicates, $\bar{x}$, as

$$\bar{x} = \sum_{i=1}^{n} \frac{x_i}{n} \tag{A-7}$$

This mean is composed of a combination of experimental numbers and is distinct from the mean value of a probability density. If each tank in a set of replicates were identical, with its parameters having the same true mean value $\alpha_i = \alpha$ and standard deviation $\sigma_i = \sigma$, then the experimental mean defined above would be the standard mean of a random sample (42). Because it is simply a combination of experimental numbers, the experimental mean has a probability density, a true mean value, and a variance associated with its probability density. Its true mean value, $\alpha_{\bar{x}}$, and variance, $\sigma^2_{\bar{x}}$, are

$$\alpha_{\bar{x}} = \mu = \sum_{i=1}^{n} \frac{\alpha_i}{n} \quad , \tag{A-8}$$

and

$$\sigma^2_{\bar{x}} = \sum_{i=1}^{n} \frac{\sigma_i^2}{n^2} = \frac{1}{n} \bar{\sigma}^2 \quad , \tag{A-9}$$

where

$$\bar{\sigma}^2 = \sum_{i=1}^{n} \frac{\sigma_i^2}{n} \quad . \tag{A-10}$$

Equation A-9 is strictly true if the x_i have Guassian probability densities, and is approximately satisfied for other probability densities.

The experimental variance, S^2, among replicates is defined as

$$S^2 = \sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{n - 1} \tag{A-11}$$

This experimental variance, S^2, is made up of a combination of experimental parameters and is distinct from the variance of a probability density. If each tank in a replicate set were truely identical, with $\alpha_i = \alpha$ and $\sigma_i^2 = \sigma^2$, the above experimental variance would be the standard variance of a sample (42).

Its true mean value (α_{S^2}) and variance (σ_{S^2}) are

$$\alpha_{S^2} = \lambda + \bar{\sigma}^2 \tag{A-12}$$

where

$$\lambda = \sum_{i=1}^{n} \frac{(\alpha_i - \mu)^2}{n - 1} \quad , \tag{A-13}$$

and

$$\sigma^2_{S^2} = \sum_{i=1}^{n} \left\{ 4(\alpha_i - \mu)^2 \sigma_i^2 + \frac{2(n-1)}{n} \bar{\sigma}^4 + 2(\sigma_i^2 - \bar{\sigma}^2)^2 \left(1 - \frac{2}{n}\right) \right\} \frac{1}{(n-1)^2} \tag{A-14}$$

We can use $x \pm S$ to indicate the range of experimental values among putative replicates; and similarily, we can use $\mu \pm \sqrt{\lambda}$ to describe the range of true mean values among putative replicates. In order to scale the experimental variance, S^2, by the precision of our experimental measurements, we form the variable

$$R = \frac{S^2}{\sqrt{\sum_{i=1}^{n} \frac{\sigma_i^2}{n}}} = \frac{S}{\bar{\sigma}} \tag{A-15}$$

Its true mean value and associated variance are given in Table A-1. To connect the variable R to a traditional probability density, we note that if the variances of the x_i were all equal ($\sigma_i^2 = \sigma^2$) but not necessarily the true mean values, α_i, then the variable $(n - 1)\, R^2$ would be described by a non-central chi-square distribution of order $n - 1$, with non-centrality parameter $(n - 1)\, \lambda/\bar{\sigma}^2$ (43,44).

The parameter λ measures the degree to which the true mean values of the putative replicates are dissimilar. If we assume the putative replicates are identical (i.e., they each have the same true mean values, $\alpha_i = \alpha$, and variances, $\sigma_i^2 = \sigma^2$), then the parameter $\lambda = 0$. Under this condition, a 90% confidence interval for the variable R is

$$0.20 \leq R \leq 1.73 \qquad \text{(A-16)}$$

If $R > 1.73$, non-zero values of λ are experimentally distinguishable from zero values and our replicate systems are statistically distinguishable from one another. Although statistically distinguishable, the differences between replicates may be such that no significant biological differences can be attributed to them.

To assess replication the following experimental variables were used: R, $S = \sqrt{S^2}$ and S/x. To assess tracking the following experimental variables were used: R, $x - x_L + S$ and $(x \pm S)/x_L$. In Table A-2 the true mean values and associated variances for the above combinations of experimental numbers are given. To compute estimates of experimental errors, we approximate the variances in Table A-1, by appropriate experimental quantities and form standard deviations by taking the square roots of the various variances. These standard deviations are multiplied by a numerical factor of 1.3 to approximate the various confidence intervals quoted in Section 3 of the main text. (For a gaussian probability density an 80% confidence interval corresponds to ±1.3 standard deviations on either side of the mean.) Table A-3 gives these errors in terms of experimentally accessible numbers for our experiments.

The various bounds on the true mean values for the combinations of experimental numbers used to assess replication and tracking can be constructed using Tables A-2 and A-3. For example, we put an upper bound on the true mean value for the parameter S.

$$\sqrt{\lambda + \bar{\sigma}^2} < S + \delta_S \qquad \text{(A-17)}$$

For the particular scale factor of 1.3 in our tables, the above inequality can be interpreted as stating to a 90% degree of confidence that the true mean value, $\sqrt{\lambda + \sigma^2}$, is less than the experimental numbers $S + \delta_S$. Similar interpretations can be made for all of our experimental numbers.

Finally, we point out that our error terms are only estimates and may be off by 10%. In stating the criteria for the degree of "goodness" of replication and tracking we took this inaccuracy into account by using more conservative requirements than we would otherwise have employed.

Table A-2

Variable	True Mean Value	Associated Variance
S	$(\lambda + \bar{\sigma}^{-2})^{1/2}$	$\frac{1}{n-1} \times \left\{ \sum_{i=1}^{n} \frac{(\alpha_i - \mu)^2}{(n-1)\cdot\alpha_{S^2}} + \beta \right\}$, where $\beta = \frac{(n-1)\,\bar{\sigma}^{-4} + \left(1 - \frac{2}{n}\right)\cdot \sum_{i=1}^{n} (\sigma_i^2 - \bar{\sigma}^{-2})^2}{2\,(n-1)\cdot\alpha_{S^2}}$
$S/\bar{x}$	$(\lambda + \bar{\sigma}^{-2})^{1/2}/\mu$	$\frac{1}{(n-1)\mu^2} \left\{ \sum_{i=1}^{n} \left(\frac{\alpha_i - \mu}{(n-1)\cdot\alpha_{S^2})^{1/2}} - \frac{((n-1)\cdot\alpha_{S^2})^{1/2}}{n\mu} \right)^2 \cdot\sigma_i^2 + \beta \right\}$
$\lvert x - x_L \rvert + S$	$\lvert \mu - \alpha_L \rvert + (\lambda + \sigma^{-2})^{1/2}$	$\left(\frac{\alpha_i - \mu}{((n-1)\cdot\alpha_{S^2})^{1/2}} + \frac{1}{n} \right)^2 \cdot\sigma_i^2 + \frac{\beta}{(n-1)} + \sigma_L^2$
$(\bar{x} \pm S)/x_L$	$(\mu \pm (\lambda + \bar{\sigma}^{-2})^{1/2})/\alpha_L$	$\frac{1}{\alpha_L^2} \left\{ \sum_{i=1}^{n} \left(\frac{\alpha_i - \mu}{((n-1)\cdot\alpha_{S^2})^{1/2}} \pm \frac{1}{n} \right)^2 \cdot\sigma_i^2 + \frac{\beta}{n-1} + \sigma_L^2\,(\mu \pm (\lambda + \bar{\sigma}^{-2})^{1/2})^2/\alpha_L^2 \right\}$

Table A-3

Variable	Error Symbol	Error Term in Terms of Experimental Quantities
S	δ_S	$\frac{1.3}{(n-1)^{1/2}} \times \left\{ \sum_{i=1}^{n} \frac{(x_i - \bar{x})^2 \cdot \sigma_i^2}{(n-1) \cdot S^2} + B \right\}^{1/2}$ where $B = \frac{(n-1)\,\bar{\sigma}^4 + \left(1 - \frac{2}{n}\right) \cdot \sum_{i=1}^{n} (\sigma_i^2 - \bar{\sigma}^2)^2}{2 \cdot (n-1) \cdot S^2}$
$S/\bar{x}$	δ_r	$\frac{1.3}{\mu(n-1)^{1/2}} \times \left\{ \sum_{i=1}^{n} \left(\frac{x_i - \bar{x}}{((n-1) \cdot S^2)^{1/2}} - \frac{((n-1)\,S^2)^{1/2}}{n\mu} \right)^2 \cdot \sigma_i^2 + B \right\}^{1/2}$
$\lvert \bar{x} - x_L \rvert + S$	Δ_S	$1.3 \times \left\{ \sum_{i=1}^{n} \left(\frac{x_i - \bar{x}}{(n-1) \cdot S} + \frac{1}{n} \right)^2 \cdot \sigma_i^2 + \frac{B}{n-1} + \sigma_L^2 \right\}^{1/2}$
$(\bar{x} \pm S)/x_L$	$\Delta_{r\pm}$	$\frac{1.3}{x_L} \times \left\{ \sum_{i=1}^{n} \left(\frac{x_i - \bar{x}}{(n-1)\,S} \pm \frac{1}{n} \right)^2 \cdot \sigma_i^2 + \frac{B}{n-1} + \sigma_L^2 \frac{[\bar{x} \pm S]^2}{x_L^2} \right\}^{1/2}$

The above expressions are valid when $S^2 \geq \bar{\sigma}^2$. When $S^2 < \bar{\sigma}^2$, or $R < 1$, replace S^2 with $\bar{\sigma}^2$ and x_i with x in the above expressions.

CRITERIA FOR REPLICABILITY

In stating criteria for the various degrees of replicability, we include effects of measurement error. We display in this section simplified upper limits to these error terms. The exact expressions are given in Tables A-2 and A-3. If a particular replicability criterion is only slightly exceeded using the simplified upper limits for the error terms, then the smaller exact expressions for the error should be considered. The term δ_S refers to error in the square root of the experimental variance, S, while the term δ_r refers to the error associated with the ratio S/x. Upper limits to them are

$$\delta_S < \frac{1.3}{\sqrt{2}} \left\{1 - \frac{1}{2R^2} + \frac{S}{\bar{x}}\left(1 - \frac{1}{R^2}\right)\right\}^{1/2} \qquad \text{(A-14)}$$

and

$$\delta_r < \frac{1.3}{\sqrt{2}} \left\{1 - \frac{1}{2R^2} + \frac{S}{\bar{x}}\left(1 - \frac{1}{R^2}\right) + \frac{2}{3}\frac{S^2}{\bar{x}^2}\right\}^{1/2} \qquad \text{(A-19)}$$

valid when $R \geq 1$; otherwise refer to Tables A-1 and A-2.

The replicability criteria are different for the two types of parameters--nutrients and phytoplankton volume densities and we describe each in turn.

Nutrients (NO_3 + NO_2 and NH_4^+)

Excellent replication (E). The condition is: $R \leq 1.73$ Within the precision of our experiments and using a 90% confidence interval, the replicate systems are not significantly distinguishable from one another or from one system sampled three times.

Good replication (G). The conditions are: $R > 1.73$ and

$$S\left(1 + \frac{\delta_S}{R}\right) \leq 0.8\ \mu M(N) \qquad \text{(A-20)}$$

The above conditions guarantee to a 90% degree of confidence that the range of true mean values of the replicates $2\sqrt{\lambda}$ is less than 1.6 μM(N). We know of no example where biological significance is attached to a 1.6 μM(N) difference between two lakes' inorganic nitrogen levels over a short period of time (16).

Adequate replication (A). The conditions are: $R > 1.73$,

$$0.8\ \mu M(N) < S \cdot \left(1 + \frac{\delta_S}{R}\right) \quad , \tag{A-21}$$

and

$$\frac{S}{\bar{x}} \cdot \left(1 + \frac{\delta_r}{R}\right) \leq 0.30 \tag{A-22}$$

The above conditions guarantee to a 90% degree of confidence that the range of true mean values of the replicates ($\mu \pm \sqrt{\lambda}$) is within ±0.3 times the true mean value (μ) of the mean among replicates.

Fair replication (F). The conditions are: $R > 1.73$,

$$0.8\ \mu M(N) < S \cdot \left(1 + \frac{\delta S}{R}\right) \leq 3\ \mu M(N) \quad , \tag{A-23}$$

and

$$0.30 < \frac{S}{\bar{x}} \cdot \left(1 + \frac{\delta_r}{R}\right) \quad . \tag{A-24}$$

Poor replication (P). The conditions are: $R > 1.73$,

$$3\ \mu M(N) < S \cdot \left(1 + \frac{\delta_S}{R}\right) \quad , \tag{A-25}$$

and

$$0.30 < \frac{S}{\bar{x}} \cdot \left(1 + \frac{\delta_r}{R}\right) \quad . \tag{A-26}$$

Phytoplankton volume densities (for individual species)

Excellent replication (E). The condition is: $R \leq 1.73$. Within the precision of our measurements and using a 90% confidence interval, the replicate systems are not significantly distinguishable from one another or from one system sampled three times.

Good replication (G). The conditions are: $R > 1.73$ and

$$\frac{S}{\bar{x}} \cdot \left(1 + \frac{\delta_r}{R}\right) < 0.30 \quad . \tag{A-27}$$

This condition guarantees to a 90% degree of confidence that the range of true values ($\mu \pm \sqrt{\lambda}$) for the replicates' phytoplankton volume densities is within ±0.3 times the true value (μ) of the mean among replicates. In comparing two lakes, a difference of 60 over a short period of time in the volume densities of the same species of phytoplankton would not be biologically significant (16,17).

Adequate replication (A). The conditions are $R \geq 1.73$ and

$$0.30 < \frac{S}{\bar{x}} \cdot \left(1 + \frac{\delta_r}{R}\right) \leq 0.70 \quad . \tag{A-28}$$

This condition guarantees to a 90 degree of confidence that the range of true mean values among replicates ($\mu \pm \sqrt{\lambda}$) is within ±.70 of the true mean value (μ) of the mean among replicates. Such order-of-magnitude estimates of phytoplankton populations often can provide adequate information about that population over a short period of time in a lake.

Poor replication (P). The conditions are: $R > 1.73$ and

$$0.70 < \frac{S}{\bar{x}} \cdot \left(1 + \frac{\delta_r}{R}\right) \quad . \tag{A-29}$$

We note that phytoplankton numbers and their volume densities can change by an order-of-magnitude over the course of a few days. Since our measurements were made weekly, some of these rapid changes can be missed in one or more of our replicates. This is an additional reason for the appropriateness of phytoplankton replication criteria that are slightly less severe than those for nutrient replication.

CRITERIA FOR TRACKING

For the quantitative analysis of tracking, we used time-aggregated quantities both for the microcosms (x_i) and the lake (x_L). We denote the true mean

value of the lake as α_L and its variance as σ_L^2. In stating criteria for the various degrees of tracking, we include the effects of measurement error. We display in this section simplified upper limits to these error terms. The exact expressions are given in Tables A-1 and A-2. If a particular tracking criterion is only slightly exceeded, then the smaller exact expressions for the error should be considered. The term Δ_s refers to error in the quantity $\bar{x} - x_L + S$; while the terms $\Delta_{r\pm}$ refer to the errors in the quantities $(\bar{x} \pm S)/x_L$. Upper limits to them are

$$\Delta_s < \frac{1.3}{\sqrt{2}} \bar{\sigma} \left\{ 1 - \frac{1}{2R^2} + \frac{S}{\bar{x}} \left(1 - \frac{1}{R^2} \right) + \frac{2}{3} \frac{\sigma_L^2}{\bar{\sigma}^2} \right\}^{1/2} \qquad \text{(A-30)}$$

and

$$\Delta_{r\pm} < \frac{1.3}{\sqrt{2}} \bar{\sigma} \left\{ 1 - \frac{1}{2R^2} + \frac{S}{\bar{x}} \left(1 - \frac{1}{R^2} \right) + \frac{2}{3} \frac{\sigma_L^2}{\bar{\sigma}^2} \frac{(\bar{x} \pm S)^2}{x_L^2} \right\}^{1/2} \qquad \text{(A-31)}$$

valid when $R \geq 1$. When $R < 1$, we set $R = 1$ in above expressions as well as in all expressions below where it appears explicitly.

Nutrients (NO_3^- + NO_2^- and NH_4^+)

Excellent tracking (E). The condition is

$$\bar{x} - x_L + S + \Delta_s \leq 3 \text{ μM(N)} \quad . \qquad \text{(A-32)}$$

The above condition guarantees to a 90% degree of confindence that the range of true mean values ($\mu \pm \sqrt{\lambda}$) for the replicates' nutrients is within 3 μM(N) of the true mean value (α_L) for the lake's nutrients, or $\mu \pm \sqrt{\lambda} - \alpha_L \leq 3$ μM(N). We know of no biologically significant differences that can be attributed to two lakes whose inorganic nitrogen levels differ by ≤ 3 μM(N) over a short period of time (16).

Good tracking (G). The conditions are

$$3 \text{ μM(N)} < \bar{x} - x_L + S + \Delta_s \quad , \qquad \text{(A-33)}$$

and

$$0.65 \leq \frac{\bar{x} \pm S \pm \Delta_{r\pm}}{x_L} \leq 1.35 \qquad \text{(A-34)}$$

The above conditions guarantee with an 80% degree of confidence that the range of true mean values of the replicates ($\mu \pm \sqrt{\lambda}$) are less than or equal to ±0.35 of the true mean value of the lake (α_L), or $0.65 \leq (\mu \pm \sqrt{\lambda})/\alpha_L \leq 1.35$. The above conditions also imply $\alpha_L \geq 8.75\ \mu M(N)$. In this range of nutrient levels a 35% difference between two lakes over a short period of time would not be perceived as significant (16).

Adequate tracking (A). The conditions are

$$3\ \mu M(N) < \bar{x} - x_L + S + \Delta_s \leq 5\ \mu M(N) \qquad \text{(A-35)}$$

and/or

$$0.30 \leq \frac{\bar{x} \pm S \pm \Delta_{r\pm}}{x_L} \leq 1.70 \quad . \qquad \text{(A-36)}$$

The above conditions guarantee to an 80% confidence level that the range of true mean values of the replicates ($\mu \pm \sqrt{\lambda}$) is less than or equal to 0.7 times the true mean value of the lake (α_L), or $0.30 \leq (\mu \pm \sqrt{\lambda})/\alpha_L \leq 1.70$.

Poor tracking (P). The conditions are

$$5\ \mu M(N) < \bar{x} - x_L + S + \Delta_s \quad , \qquad \text{(A-37)}$$

and

$$\frac{\bar{x} - S - \Delta_r}{x_L} < 0.30 \quad , \qquad \text{(A-38)}$$

and/or

$$\frac{\bar{x} + S + \Delta_{r+}}{x_L} > 1.70 \qquad \text{(A-39)}$$

Phytoplankton volume densities (for one species). To motivate our choice of phytoplankton tracking criteria, we note that typically in lakes, over time intervals of several weeks, 1 to 3 species of phytoplankton dominate by volume or numbers, while up to two orders of magnitude of additional species are present in very low, and often difficult to detect, numbers. The dominant species as well as those present in trace amounts, will change with time. Only the dominant species present in each interval are considered in our assessment of tracking.

Excellent tracking (E). The condition is:

$$0.35 \leq \frac{\bar{x} \pm S \pm \Delta_{r\pm}}{x_L} \leq 1.65 \quad . \qquad \text{(A-40)}$$

This condition guarantees with an 80% degree of confidence that the range of true mean values ($\mu \pm \sqrt{\lambda}$) for the replicates' phytoplankton volume densities is $\leq$ 0.35 times the true mean value (α_L) of the lake's phytoplankton volume density, or $0.35 \leq (\mu \pm \sqrt{\lambda})/\alpha_L \leq 1.65$.

Good tracking (G). The condition is

$$0.30 \leq \bar{x} \pm S \pm \Delta_{r\pm} \leq 1.70 \qquad \text{(A-50)}$$

This condition guarantees to an 80% degree of confidence that the range of values for the replicates' ($\mu \pm \sqrt{\lambda}$) photoplankton volume density is ±0.7 times the true mean value (α_L) of the lakes phytoplankton volume density, or $0.3 \leq \mu \pm \sqrt{\lambda})/\alpha_z \leq 1.70$. This level of agreement between two different systems (natural vs laboratory) is often better than that observed in a single natural lake between two successive years (16,17).

Fair tracking (F). The condition is:

$$0.25 \leq \frac{\bar{x} \pm S \pm \Delta_{r\pm}}{x_L} \leq 4 \qquad \text{(A-51)}$$

This condition guarantees to an 80% confidence level that the range of true mean values ($\mu \pm \sqrt{\lambda}$) in the replicates are within a factor of 4 of the lake's true mean value (α_L).

Poor tracking (P). The conditions are:

$$\frac{\bar{x} - S - \Delta_r}{x_L} < 0.25 \qquad \text{(A-52)}$$

and/or

$$4.0 < \frac{\bar{x} + S + \delta_{r+}}{x_L} \qquad \text{(A-53)}$$

Our criteria for replication and tracking are quite stringent. For both replication and tracking, we include the measurement error of each experimental quantity or combination of quantities in such a way as to construct the worst possible case. In other words, our error terms are taken so as to make satisfying any given "goodness" criteria most difficult. This even includes assigning an error to the experimental variance, S^2, which many researchers use without quoting the precision of its determination, or realizing that it is an experimental number subject to measurement uncertainties. Our analysis of replicability allowed the parameters in each of the putative replicates to have true mean values, α_i, and associated standard deviations, σ_i, which could differ from microcosm to microcosm, in contrast to traditional analyses which assume that the parameter in each replicate has the same true mean value ($\alpha_i = \alpha$) and standard deviation ($\sigma_i = \sigma$). The effect of this is to make more stringent the tracking criteria, for we required that not only the true mean value among replicates, μ, be sufficiently near the lake's true mean value, α_L, but that the whole range of replicate true mean values ($\mu \pm \sqrt{\lambda}$) be near the lake's true mean value (α_L). In short, we required both sufficiently good replication and tracking for good tracking to be claimed.

APPENDIX B

EXPERIMENTAL DATA FOR EXPERIMENTS I - IV

The data for each experiment are presented sequentially, beginning with phytoplankton, followed by nutrients and zooplankton. A dash in the data body indicates no measurement was taken. Where no datum value is presented, none of that particular parameter was present on that day.

EXPERIMENT I

Phytoplankton $\frac{\mu^3}{ml} \times 10^6$		1 5/26/78	7	14	21	28	35	42	49	56	63	77
Ankistrodesmus	A	.050 .053 .053		.056	.038						.0099	
	B	.10 .083 .099										
	C	.076 .11 .14	.0024 .0026 .59	.048	3.0 .60 .89	.46						
	D	.12 .00058 .12	1.2 .0036 .69		 .50	 .33						
	E	.014 .089 .018	.53 .73 .53		1.0 .092	.36 .16	.086		.46 -		.36	1.5 .059 5.6
LRGT's	A		.034									
	B			.064 .84 .048								
	C				.11				.056			
	D			.024								
	E								-			

		1 5/26/78	7	14	21	28	35	42	49	56	63	77
Flagellate I	A											
	B											
	C											8.4
	D											2.4
	E			.022					-			
Flagellate II	A											
	B											
	C			.30 .74								
	D			.48								
	E			.56					-			
Unidentified Flagellate	A				.036 .025							
	B				.10							
	C											
	D											
	E								-			

		1 5/26/78	7	14	21	28	35	42	49	56	63	77
Quadrigula	A											
	B											
	C							.53	.21	.60 .92	1.0	
	D									.76 .50	.69 .73	22 15
	E											2.2
Scenedesmus	A											
	B											
	C											
	D											
	E											.66 .021
									-		1.6	
Kirchneriella	A											
	B											
	C										50	
											.36	1.1
	D										5.6	
	E								-			

		1 5/26/78	7	14	21	28	35	42	49	56	63	77
Phacus	A										.060	.047 .13
	B											.077
	C											
	D											
	E								-			
Unknown I	A											
	B											
	C											.20 .070
	D											
	E								-			
Schroderia	A											
	B										.013	.99
	C											
	D								.076		.17	2.5
	E								-			

		1 5/26/78	7	14	21	28	35	42	49	56	63	77
Gloeocystis	A											
	B											
	C										.14	
	D											
	E								-			
Small blue-green	A	.066 .095 .22	1.3 1.0 .95	1.4 1.1 .88	.024 .30 .43		.0060					
	B	.10 .093 .22	.45 .98 .14	2.1 1.8 1.7	.75 .40 .43							
	C	.35 .28 .24	.40 .67 2.0	5.6 .46	.63							
	D	.33 .11 .30	1.6 .52 .17	.10 .10								
	E	.33 .29 .23	.70 1.3 1.6	.17 .38	.013				-			
Anabaena sp.	A										.022 .0011	
	B											
	C											
	D								.0031			
	E						.0094		-			

		1 5/26/78	7	14	21	28	35	42	49	56	63	77
Cryptochrysis	A											
	B											
	C		.000029									
	D		.032									
	E		.041						-			
Closterium	A									.31	1.0	
	B								1.0	7.4	20 11	52 44
	C					.31	1.6	8.1	1.0 7.7		11	
	D									3.3	14	36
	E										18 27	75 62
Synedra radians	A			.00036		.13 .040	.12 .029 .068	.086	.10 .16	.017	.017	
	B			.0088								
	C					.086						
	D			.00034 .00026 .00026	.00014 .38							
	E			.00096 .0032 .0022		.12 .017 .21	.046 .023 .029	.46 .15 .15	-			

		1 5/26/78	7	14	21	28	35	42	49	56	63	77
Mougeotia	A				.010			.044	.16	.011		.060
							.14	2.3	.48	.022		
				.013		.014	.18	2.8				.042
	B					.064	13	.23	.025			.055
						.096	13	6.5	.20	.022		
				.0028		.051	11	5.8	.61	.061	.0062	
	C				.018	.45	13		6.6	3.2	.21	
				.010		3.6	12	11	4.4	.10		
					.017			.26	.55	.19	.065	
	D			.0056	.13	19	5.0	.00083	.033	.025		
				.0072	.015	10	3.1	.090	.013	.020		
				.0026		8.1	3.1	.39	.058	.091		
	E			.0018	.016		.0084	.022	.15	6.5		
				.0050	.0034	.14	.014	.25	.12	5.1	.28	
					.0068	.15	.0039		-	.071		

		1	7	14	21	28	35	42	49	56	63	77	84	91
		5/26/78												
NH_4^+	A	3.9	1.9	-	2.5	2.0	3.5	4.0	3.8	3.0	2.5	-	5.4	56.1
(μM)		3.2	4.7	-	2.3	2.8	3.5	3.6	3.7	2.0	9.3	-	2.6	95.9
		4.0	2.6	-	2.4	1.8	2.8	3.1	3.5	2.1	6.9	-	2.7	5.6
	B	4.3	3.0	-	2.2	3.5	3.6	4.0	4.1	2.4	2.5	-	3.7	12.8
		3.8	3.2	-	2.2	3.7	3.7	4.4	4.7	2.5	3.3	-	2.8	20.3
		1.9	1.9	-	2.4	3.9	3.0	4.0	3.8	1.9	4.0	-	3.8	50.1
	C	6.1	2.3	-	1.5	2.1	2.1	3.5	3.4	3.4	3.3	-	1.4	27.1
		2.5	1.9	-	2.1	4.8	3.4	2.5	2.8	1.9	2.3	-	2.4	19.2
		2.4	2.1	-	1.2	1.9	3.6	2.8	3.2	4.1	1.9	-	1.1	20.4
	D	8.7	2.3	-	1.5	2.7	2.8	3.1	3.4	6.5	5.7	-	2.7	3.9
		6.4	1.9	-	2.3	1.8	3.2	3.1	3.4	2.0	5.1	-	2.4	10.2
		2.2	2.2	-	2.1	1.4	2.5	9.3	3.2	2.3	2.7	-	1.9	2.7
	E	2.6	1.7	-	2.3	1.5	2.5	2.8	3.5	2.5	5.3	-	2.7	1.5
		2.8	1.9	-	5.6	3.5	2.8	3.0	3.2	16.8	4.5	-	4.3	5.5
		4.4	2.5	-	2.4	1.8	3.2	3.0	2.9	4.1	2.2	-	2.2	5.0
$NO_3^- + NO_2^-$	A	-	3.1	-	2.2	3.5	4.2	1.9	1.8	3.1	2.4	-	6.5	7.9
		-	15.1	-	1.8	3.9	6.6	1.3	1.8	2.3	24.3	-	2.9	6.4
(μM)		-	13.3	-	1.3	1.9	5.0	1.3	2.2	3.0	27.6	-	4.8	3.4
	B	-	7.1	-	1.3	2.7	3.0	1.6	1.4	2.5	3.5	-	4.0	2.6
		-	-	-	1.3	2.0	7.7	1.6	2.3	2.8	9.1	-	4.0	3.3
		-	6.2	-	1.8	4.7	5.0	2.3	2.1	1.9	8.6	-	5.5	4.9
	C	-	7.5	-	1.8	2.4	5.4	1.6	3.1	-	3.4	-	6.3	3.6
		-	6.6	-	1.3	3.7	5.8	1.3	6.3	3.0	5.8	-	7.0	3.6
		-	6.2	-	1.8	3.0	5.6	2.6	4.2	3.1	13.2	-	6.9	3.3
	D	-	5.8	-	1.8	2.1	3.2	1.9	3.5	3.4	15.4	-	4.3	2.5
		-	8.4	-	1.8	2.9	-	3.0	3.4	3.8	24.1	-	7.7	3.3
		-	6.2	-	1.8	2.7	4.9	2.3	3.8	2.9	14.0	-	6.1	1.9
	E	-	5.8	-	1.8	2.7	4.4	1.9	4.0	3.5	8.2	-	5.9	2.8
		-	6.2	-	1.3	2.5	3.8	1.6	4.0	2.6	2.9	-	9.2	3.1
		-	6.2	-	2.7	2.2	4.8	1.9	-	2.8	4.3	-	5.4	3.3

		1	7	14	21	28	35	42	49	56	63	77	96
		5/26/78											
Zooplankton													
$\frac{\mu^3}{100ml} \times 10^6$													
Protozoa	A		-										
	B		-										
		.20		.065									
	C								.080	2.7		.85	.99
			1.8							.13			30
	D	.065											
								.18		.19			
		.065	.39							.064			
	E	.065								-			
			6.5										
			.065					1.1					
Rotifera	A	.14	.99	3.0	1.2	39	9.4	6.6	8.9	29	13	20	8.9
		3.1	-	6.1	2.4	16	6.7	3.3		1.2	1.2	2.5	.12
		.99				17	2.2	.099	3.2	20	6.7	4.6	4.5
	B	.28	.99	13		57	3.2	22		4.1	4.6	1.4	.99
		.14	-	8.1	7.2	32	27	7.1	5.1	2.2	4.8	.12	5.5
		.28		2.0		2.4	.99	1.4	.099		1.2		5.9
	C	.14	.14	9.9		.34		2.1	2.0	2.0			
			.14	3.8	12	13	3.4		.76		1.8		
			.84	2.0	7.9	2.4	5.4	1.6	4.3	.14	2.2	.099	
	D	.42	.14	5.9	.28			3.0	20	39	48	77	5.9
		.28	.14	8.9	8.9	.20	3.3	19	24	27	24	21	29
		1.3	.14	3.0	2.3	12	12	7.9	4.1	8.3	32	91	61
	E	1.3	14	.99		2.9	6.6	17	66	-	3.6		49
		.14	3.5	7.9	5.0	1.5	3.6	5.0	83	130	57	2.0	3.6
		.28	.28	2.0	2.0	2.2	6.2	4.7	81	11	3.2	3.0	11

		1 5/26/78	7	14	21	28	35	42	49	56	63	77	96
Copepoda	A				1.2	7.2	6.0		8.4	18	24	28	37
			-		1.2	4.8	6.0	4.8	35	1.2	4.8	12	7.2
					2.4	11	12	14	14	37	20	85	26
	B						1.2		7.2	4.8	28	13	9.6
			-			1.2	1.2	2.4	1.2	23	17	14	12
							2.4	2.4	4.8	2.4	13	3.6	
	C							1.2					
													1.2
	D					1.2			1.2	1.2		7.4	40
					2.4						9.8	7.2	6.0
								100	2.4	1.2		140	39
	E				1.2	3.6		20	25	-	88	38	
								1.2	14	20		40	16
									4.8	35	2.4	58	1.2
Cladocera	A	30	60		640	190	140	210	64	350	450	150	100
			-	12	360	90	110	150	46	52	360	72	110
		12			460	320	160	69	30	37	370	570	340
	B	24			71	290	42	330	37	420	470	180	100
			-	59	400	250	400	610	14	4.7	160	37	37
		6.0			960	190	230	87	160	37	390	93	450
	C		14		20	310	770	660	1100	500	1000	340	290
				14	9.4	480	570	800	440	1300	730	200	200
				9.4	290	510	380	500	470	430	460	36	260
	D			100	450	200	750	690	180	890	360	400	950
				96	840	360	710	740	740	510	760	940	6.0
				36	97	450	410	640	1000	330	230	280	18
	E	6.0	54	6.0	240	190	170	550	910	-	410	260	320
				21	560	290	570	190	710	380	550	670	790
					650	600	500	900	540	200	540	220	510

EXPERIMENT II

		0	7	13	19	26	33	41	48	55	61	78	85	92
		10/19/78												
Phytoplankton $\frac{\mu^3}{ml} \times 10^6$														
Fragilaria	A	-	.22	.12						.014				
		-	.063	.077										
		-	.035	.077										
	B	-	.15	.16	.014									
		-	.084	.11										
		-	-	.17	.0070									
	C	-	.13	.084										
		-	.13	.056										
		-	.091	.049										
	D	-	.014											
	E	-	.091		.014									
(numbers represent dublicate lake samples)	F	-		.028										
	Field	.063	.34	.89	1.3	4.8	1.6	.67	.15	.18	.084	.16	-	.028
		.063	.25	.76	1.5	6.2	1.9	.34	.13	.16	.056	.12	-	.042

		0 10/19/78	7	13	19	26	33	41	48	55	61	78	85	92
Stephanodiscus	A	- - -												
	B	- - -	.014											
	C	- - -												.0096
	D	-												
	E	-												
	F	-												
	Field				.00091 .0016	.096 .062				.038 .067	.072 .067	.72 1.1	- -	3.0 3.0
Asterionella	A	- - -												
	B	- - -	-											
	C	- - -												
	D	-												
	E	-												
	F	-												
	Field										.070 .055	.65 1.5	- -	2.0 2.1

		0 10/19/78	7	13	19	26	33	41	48	55	61	78	85	92
Coscinodiscus	A	- - -												
	B	- - -	- 											
	C	- - -							2.0 .49	1.8 .61 2.3	3.1 .37 .24	7.4 .24 .37	1.8 .24 .37	
	D	-								.36	.49	.24		
	E	-												
	F	-										.12		
	Field												- -	
Ceratium	A	- - -	.10 .52 .42											
	B	- - -	.31 .42 -											
	C	- - -	.21 .21 .21											
	D	-	.10											
	E	-	.42	.21										
	F	-	.10											
	Field	 1.4	.31 .52	.21 .11				.21 .10	.20 .21	.52 .52	.42 .10	.10 .11	- -	

		0	7	13	19	26	33	41	48	55	61	78	85	92
		10/19/78												
Unid.														
Flagellate I	A	-	.026											
		-	.0050										.0066	.24
		-	.033								.0026		.039	.38
	B	-	.0031							.056	.32	.056	.013	
		-	.12											.18
		-	-								.053	.34	.27	.035
	C	-	.050					.022	.22	1.7	.39	.090	.67	.12
		-	.080					.021	.081	.14	.16	.31	.24	.11
		-	.049						.22	.56	.18	.89	.23	.33
	D	-	.049								.049		.0088	
	E	-	.056										.014	.060
	F	-	.011								.084	.13	.092	
	Field												-	
													-	
Unid.														
Flagellate II	A	-	.087											
		-	.082											
		-	.15											
	B	-	.13											
		-	.053											
		-	-											
	C	-	.18											
		-	.84											
		-	1.2											
	D	-	.14											
	E	-	.082											
	F	-	.18											
	Field		.11	.044										
		.056	.090	.023										

		0	7	13	19	26	33	41	48	55	61	78	85	92
		10/19/78												
Unid.														
Flagellate III	A	-												
		-												
		-					.0038							
	B	-												
		-												
		-	-											
	C	-		.018		.056								
		-		.018										
		-		.011										
	D	-												
	E	-												
	F	-									.035			
	Field												-	
													-	
Unid.														
Flagellate IV	A	-						.0095						
		-					.0027	.018						
		-						.013						
	B	-												
		-												
		-	-				.00057							
	C	-												
		-					.0020							
		-					.00032							
	D	-					.00076							
	E	-				.0074	.0028							
	F	-												
	Field												-	
													-	

		0	7	13	19	26	33	41	48	55	61	78	85	92
		10/19/78												
Unid.														
Flagellate V	A	-												
		-							.062					
		-												
	B	-												
		-												
		-	-											
	C	-						.0017						
		-						.0068						
		-												
	D	-												
	E	-							.0025					
	F	-												
	Field												-	
													-	
Phacus	A	-									.0028			
		-								.037	.012		.0037	
		-								.012				
	B	-												
		-												
		-	-							.0047	.0093			
	C	-												
		-												
		-												
	D	-								.012				
	E	-						.0093	.060	.041	.11	.026	.033	.060
	F	-												
	Field								.0056	.0037	.022		-	
									.0037	.010	-		-	

		0	7	13	19	26	33	41	48	55	61	78	85	92
		10/19/78												
Filamentous green I	Field			.0073									-	
													-	
Filamentous green II	A	-					.096	.74	1.2	.040	.018			
		-						.27	.51	.74	1.8	.85	1.8	.078
		-							.081	2.3	.022	.37	.22	1.6
	B	-												.11
		-												
		-												.020
	C	-						.48						
		-						.065	.071					.055
		-						.24	.19	.015	.32	.12	.020	
	D	-					.46	.97	1.3	.34	.25	.95	1.1	.71
	E	-						.029	.0033				.013	
	F	-						.065	.033	.025		.0066		.20
	Field			.017									-	
													-	
Unid.														
Blue green I or bacterium	A	-												
		-												
		-												
	B	-												
		-												
		-	-										.26	.067
	C	-												
		-												
		-												
	D	-												.020
	E	-												
	F	-												
	Field												-	

		0	7	13	19	26	33	41	48	55	61	78	85	92
		10/19/78												
Unid.														
Blue green II	A	-		.14	.012	.016					.0093			
		-		.040	.022	.028	.022							
or bacterium		-		.050	.062	.0062			.040					
	B	-		.26	.099	2.1	5.8	5.2	1.6	.11				
		-		.037	.62	3.2	5.2	5.5	3.7	.39	.0093			
		-	-	.025	.0031	.0031		.030	.0079		.0031			
	C	-			.021	.022	.031							
		-			.022	.025								
		-			.0093	.0093	.0031							
	D	-		.79	.0062	.016	.043							
	E	-		.51	.019		.0031							
	F	-		.068	.016			.062	.078	.070				
	Field												-	
													-	
Anabaena	A	-												
		-												
		-												
	B	-												
		-	.053											
		-	-	.091										
	C	-	.15											
		-	.21											
		-	.20											
	D	-	.091	.70										
	E	-	.30	.22										
	F	-	.31											
	Field		.13	.053	.20	.15	.11	.64	.17	.084		.0076	-	
			.091	.17	.23	.12	.14	.71	.053	.030		.015	-	

		0	7	13	19	26	33	41	48	55	61	78	85	92
NH_4^+	A	-	5.3	4.0	3.5	2.7	2.5	1.6	1.5	2.2	2.5	2.9	-	3.2
		-	7.8	4.5	3.5	2.8	3.3	1.6	1.5	2.3	2.3	2.7	-	3.4
(μM)		-	5.7	4.1	3.0	2.8	3.3	1.5	1.2	2.2	2.1	2.9	-	3.6
	B	-	8.4	8.4	5.2	2.5	3.8	2.4	1.2	5.2	5.8	6.4	-	7.3
		-	7.9	6.4	6.7	2.5	3.5	1.8	1.3	4.2	5.8	7.3	-	4.2
		-	6.6	4.4	7.9	8.6	9.6	3.6	2.7	3.8	2.4	3.2	-	3.3
	C	-	6.1	4.0	3.4	2.9	3.5	2.1	1.5	2.4	2.2	2.9	-	3.2
		-	6.1	3.4	2.9	2.7	3.5	1.7	1.3	2.2	1.9	2.9	-	3.0
		-	5.3	2.9	2.9	2.1	2.9	1.6	1.3	2.2	1.8	2.8	-	3.5
	D	-	4.5	3.4	3.0	2.8	4.1	1.5	2.3	3.2	2.2	2.9	-	2.3
		-	5.0	3.3	2.7	2.8	3.0	1.6	1.6	3.0	2.5	3.2	-	3.9
		-	4.2	2.9	2.9	3.2	3.5	1.6	1.3	3.0	2.8	3.4	-	4.0
	E	-	3.9	2.9	3.5	3.5	4.1	1.9	2.1	4.2	4.1	4.1	-	5.1
		-	4.1	4.0	4.6	5.8	5.5	1.3	2.5	4.7	3.6	5.1	-	6.3
		-	3.4	3.3	4.5	4.7	5.3	2.8	3.4	4.4	4.2	5.3	-	5.7
	F	-	4.5	4.0	3.6	3.0	3.4	1.3	1.5	3.5	3.0	3.6	-	3.8
		-	3.3	3.3	2.7	2.8	2.9	1.7	1.3	3.0	2.3	2.8	-	4.5
		-	3.4	2.4	3.4	2.9	3.3	1.7	1.5	4.7	3.2	3.3	-	3.8
	Field	-	4.2	3.3	2.9	3.8	5.3			11.8	14.7	14.7	-	6.7
		-	3.9	4.1	3.4	4.0	5.9			13.8	16.0	14.4	-	7.3
	av.	-	4.1	3.7	3.2	3.9	5.6	9.0	12.1	12.8	15.4	14.6		7.0

		0	7	13	19	26	33	41	48	55	61	78	85	92
$NO_3^- + NO_2^-$	A	-	3.1	2.8	4.2	2.7	3.1	-	-	4.5	6.6	4.7	-	3.8
		-	4.0	3.9	4.4	2.6	2.9	-	-	4.3	3.1	4.9	-	3.1
(μM)		-	3.3	3.5	3.6	2.8	2.6	-	-	4.7	2.7	4.6	-	3.6
	B	-	3.5	4.2	6.4	4.7	2.6	-	-	6.4	4.6	8.0	-	10.5
		-	3.5	3.5	5.4	3.8	2.8	-	-	4.0	4.3	7.0	-	8.5
		-	3.5	3.4	5.8	5.3	5.4	-	-	7.9	8.9	4.9	-	5.0
	C	-	3.5	2.8	4.5	2.8	3.1	-	-	5.0	3.2	3.3	-	1.9
		-	3.5	3.1	3.9	2.9	3.1	-	-	3.3	2.8	4.3	-	4.0
		-	3.0	2.2	3.8	2.9	3.0	-	-	3.6	3.7	4.3	-	4.0
	D	-	4.2	3.0	4.6	3.5	7.2	-	-	3.5	6.8	5.5	-	10.0
		-	3.6	2.6	3.9	3.0	6.4	-	-	3.3	4.1	6.6	-	4.2
		-	4.2	2.4	4.7	2.5	7.4	-	-	3.8	3.6	5.4	-	4.0
	E	-	4.0	2.7	5.0	5.5	16.3	-	-	14.6	14.6	19.3	-	22.5
		-	3.1	2.7	4.7	4.9	14.4	-	-	11.8	14.0	21.7	-	22.2
		-	3.6	2.6	5.6	5.4	19.7	-	-	16.6	22.3	24.2	-	33.2
	F	-	4.7	3.1	4.4	3.1	7.2	-	-	4.5	5.0	5.4	-	4.8
		-	3.2	2.6	4.3	2.7	6.5	-	-	4.1	3.6	5.4	-	3.9
		-	4.0	2.4	4.3	3.1	6.2	-	-	4.6	4.7	4.1	-	4.7
	Field	-	3.1	2.8	4.1	3.3	6.2	-	-	5.0	4.7	8.2	-	8.3
		-	2.7	2.2	3.5	2.9	6.7	-	-	5.2	5.3	9.3	-	9.3
	av.	-	2.9	2.5	3.8	3.1	6.5			5.1	5.0	8.8	-	8.8

Zooplankton $\frac{\mu^3}{100\ ml} \times 10^6$		0	7	13	19	26	33	41	48	55	61	78	85	92
Protozoa	A			.0088 .0050										
	B			.0092 .0034 .0025		.00084	.018 .017 .00042	.0038 .00084	.00042 .011	.0042	.012 4.2	.0042 .0016		.0063
	C			.0017 .0012 .00042						-				-
	D		.038	.0029										-
	E		.021	-				.0034			.00042	.00084		
	F		.0055	.0055										
	Field	.0054											-	
Rotifera	A		77 42 43	24 18 20	2.0 .62 .62	4.2 4.1 2.2	2.5 1.8 .69	4.4 7.0 .37	3.2 10 .98	.11 1.6 .98	.74 .14 .11	.33 5.8 3.2	.14 1.6 3.4	.37 .42 .14
	B		78 20 49	17 37 22	1.9 1.9 7.9	.37 .64	1.5 .74 .33	.37 .14	3.2 .74 1.6	 2.6	1.6	.28 .11	2.6 .73	9.3 .14
	C		12 24 6.7	17 17 9.4	6.3 9.0 6.3	8.3 8.3 97	4.2 3.3 4.6	2.7 2.4	.37	-	3.1	.98 .23 .11	1.2 .12 .70	3.9 2.3 -
	D		30	110	.88	2.4	7.9	13	3.8	8.0	6.3	7.4	12	-
	E		30	-	.97	2.0	.14	1.3	.14	.14	.28		12	
	F		80	170	3.6	7.6	9.5	16	2.3	2.2	5.6	1.2	1.9	1.5
	Field	.94	32	11	1.2	1.3	11	.70	1.7	12	8.5	.70	-	

		0	7	13	19	26	33	41	48	55	61	78	85	92
Cladocera	A			1200	610		160	9.2	4.6	12	9.2			
				48		160	75	71	29	18	23	42	1.4	36
				64	320	450			8.2	44	6.9		12	24
	B			28	160				4.6		3.7	34	670	290
				16	140		320	830		160	26	1.4	170	95
				16	800	610	80	2600	2.3	13	170		46	1.4
	C			290	160	200	1.4	820	25	14	17	25	36	15
				780	770	200	9.7	300	200	12	52	23	260	100
				670	29	3.7	00	14	6.9	-	6.9	2.3		-
	D			33	510	3400	2300		12	4.6	3.9	19	4.6	-
	E			-	690	2000	1200		160	160	2.3		4.6	2300
	F			160	490	6.4	290		2.8	12		2.8		
	Field	80	8.0					.70	80			12	-	
Copepoda	A		1.2	8.1	4.8	4.8	200	100	2.4	2.4	3.6	3.6	24	8.6
				7.0	320	130	2.4	8.4	7.2	11				
			130	3.6					3.6	1.2	1.2	.45		
	B		1.2		63	3.6	18	160	2.4	210	85	74	59	2.4
			63	7.0	8.4	810	70	40	3.6	4.8	140	11		9.8
				2.4	63	3.6	6.0	7.0	8.4	3.6		18		
	C			3.6	35	100	3.6		3.6	2.4	63	8.4	2.4	18
				67		100	2.4	14	12	30	28			140
				1.2	1.2	8.4	110	1.2	110	-	2.4	22	2.4	-
	D				3.6		200	65	48		1.2			-
	E		63	-	110		1.2	1.2	1.2		4.8	35		17
	F					100	43	7.0	150	7.2	310		1.2	200
	Field		53	110	100	1.8	66	32	30	7.4	8.5	12	-	18

EXPERIMENT III

	day:	1	7	14	21	28	35	42	49	56	63	70	77
		4/20/79											
PHYTOPLANKTON $\frac{\mu^3}{ml} \times 10^6$													
Stephano-discus	A	--	1.1	1.3	.30	.017	.0034	.0068			.037		
		--	1.3	1.7	.52	.15							
	B	--	1.1	1.2	.33	.024	.00034						
		--	1.3	1.8	.20								
		--	.92	2.3	.33	.092	.0034						
	C	--	.97	1.4	.50	.18	.13						
		--	1.2	2.2	.48	.19	.078						
		--	1.4	2.1	.20	.0068							
	D	--	1.1	1.3			.014						
		--	1.3	1.8	.46	.020							
Synedra		--											
	A	--											
		--											
		--											
	B	--											
		--											
		--											
	C	--											
		--											
		--											
	D	--											
		--											
		--											
	Field	--											.049
		--											

Phacus Flagellates	A	--	.073			.013	.053	.036	.097			.31		
		--				.024	.031	.0019	.047	.057			.040	
	B	--				.034	.031	.0017				.089		
		--				.0043	.078						.069	
		--		.133		.024	.052			.080	.055	.025	.057	
	C	--		.136		.051	.022	.037		.020		.012	.035	
		--		.13		.023	.054	.070		.010	.31			
		--		.121			.016	.0110	.054					
	D	--			.018	.017	.047		.090	.16	.089	.40	.35	
		--				.0123	.043	.011						
	Field		.078			.0059		.052	.048	.41	.36			
Ceratium	A	--												
		--												
	B	--												
		--												
		--												
	C	--							.10					
		--						.21	.10					
		--												
	D	--												
		--												
	Field						.10	.10	.10					
Ulothrix	A	--												
		--										.22	.074	
	B	--							.035	.32	1.1	1.2	1.4	
		--						1.0	1.4	4.6	2.3	.44	.047	
		--							.090	.031	.059	.14	3.9	
	C	--							.020	.024	.020	.024	.012	
		--							.016		.0039	.12	.70	
		--							.58	3.7	9.1	1.6	.37	
	D	--								.016	.063	.063	.28	
		--									.024	.020		
	Field													

Oscillatoria Anabaena sp.	A	--											
		--									.00084		
	B	--											
		--											
		--						.047					
	C	--							.00076		.00108		
		--						.00075			.00044	.013	
		--											
	D	--							.13				
		--							.00025	.0055	.00042		.045
	Field							.011		.019	.11	2.7	.12
Gloeocystis LRGT's	A	--						.0018	.050	.29		.80	1.9
		--											
	B	--							.0047				
		--											
		--											
	C	--									.0049		
		--							.0030				
		--											
	D	--			.089	.046							
		--											
	Field												
NH_4^+ (μM)		6.8	5.0	4.1	4.0	2.9	4.3	2.9	4.3	2.9	3.3	2.6	2.7
	A	5.8	4.3	4.4	4.9	3.7	3.9	3.1	3.7	2.5	2.4	2.6	3.1
		7.0	4.1	3.5	4.2	3.6	3.9	3.2	4.1	2.8	3.0	2.8	2.8
		6.5	5.4	3.9	5.0	5.2	5.4	5.5	6.4	5.0	5.1	5.4	5.2
	B		5.5	4.8	5.5	5.3	5.5	5.9	6.1	4.7	2.2	2.6	3.3
			5.7	5.3	5.8	5.6	6.4	4.1	2.5	2.1	2.0	2.3	2.8
			6.8	2.8	4.9	4.1	3.7	2.9	2.9	2.6	3.6	3.8	3.0
	C		6.8	4.2	4.0	4.1	4.6	4.6	4.8	4.7	5.8	6.4	7.1
			7.3	4.2	3.7	4.6	4.5	3.5	4.7	5.9	6.3	6.9	8.3
			7.9	5.1	5.1	4.1	4.5	4.4	2.8	2.2	2.1	2.5	2.4
	D		8.0	4.7	5.1	3.1	2.8	2.3	2.7	3.2	4.4	3.3	2.8
			6.7	3.6	6.6	7.0	7.1	7.5	10.0	7.9	7.8	8.0	5.8
	Field		8.4	3.3	4.3	3.4	3.2	2.6	3.6	2.8	2.7	2.9	3.2
			5.7	3.1	3.5	2.9	3.0	3.4	3.4	2.9	3.1	2.9	3.2
		av.	7.1	3.2	3.9	3.2	3.1	3.0	3.5	2.9	2.9	2.9	3.2

$NO_3^- + NO_2^-$ (μM)													
		3.8	4.1	5.0	2.7	5.3	5.6	6.4	10.1	10.3	15.0	12.4	17.8
	A	3.4	4.3	3.5	6.0	6.3	7.3	6.2	9.8	10.2	11.5	9.4	9.3
		3.5	4.2	3.4	3.7	3.9	9.6	5.6	9.9	10.6	14.9	13.6	14.4
			2.9	4.2	2.8	3.7	8.0	3.8	4.8	5.2	11.6	8.8	6.0
	B		3.1	2.3	2.6	5.6	3.1	3.8	4.5	5.4	8.0	6.2	4.9
			6.0	2.0	2.9	2.4	6.4	2.8	3.2	3.0	2.7	2.7	1.4
			5.1	3.6	2.6	4.5	3.6	2.7	3.3	2.0	4.9	3.7	4.2
	C		5.6	3.8	2.1	3.7	2.8	4.4	3.7	3.5	8.8	6.0	5.0
			3.8	2.6	3.0	2.7	2.7	3.5	3.7	4.1	6.7	8.4	6.9
			4.8	2.9	3.7	8.1	4.3	6.1	5.9	4.7	6.0	3.6	3.2
	D		3.1	3.6	5.1	5.9	4.7	1.9	1.1	1.6	3.7	3.5	2.7
			2.6	2.9	2.0	6.7	3.0	4.2	3.9	3.8	6.5	9.1	5.4
	Field		1.7	1.3	2.5	2.0	0.84	0.97	0.91	1.0	2.0	3.6	3.0
			2.2	1.3	1.8	1.2	0.97	1.5	1.1	1.3	2.2	2.2	1.6
		av.	2.0	1.3	2.2	1.6	0.91	1.2	1.0	1.2	2.1	2.9	2.3

ZOOPLANKTON

$\frac{\mu^3}{100\ ml} \times 10^6$

Protozoa	A	--											
		--	.00084										
	B	--	.00084										
		--	.00084			.12							
		--	.0021										
	C		.0021										
		--	.0025										
		--	.0013										.00042
	D		.0013										
		--	.0034										
	Field				.00021		.0013						
Rotifera	A	--	9.3	2.8	14	.51	1.9	1.1	.37	.74		.51	.14
		--	6.1	6.2	6.9	1.0	.14	.32	.48	1.2	51	.28	.65
	B	--	6.5	6.2	12	2.7	.74	.88	.42	.39	.39	1.7	3.0
		--	5.7	4.3	3.0	1.3		.62	.51	1.6	1.4	1.1	.28
		--	6.8				.48		.28				
	C	.69	5.2	19	2.8	3.0	1.2	1.5		1.9	2.1	1.1	1.5
		--	6.0	15	3.0	.63	.69	5.2	.14	2.1	.51	1.9	.14
		--	6.2	15	5.3	.74		2.1	.88	.14	.37	.56	8.8
	D	2.4	5.2	2.8			9.6						
		--	3.8	36	15	3.3	1.8	1.4	.88		.11		
	Field	3.7	1.5	1.1	7.9	3.4	3.2	1.3	.37	.07	.70	.35	.35

Copepoda	A	--	160	40	1.2	16		19	2.4	4.8			1.2
		--	19	18	6.2	37	.45	9.8		1.2	.60	6.0	6.0
	B	--	17	51	64	1.2		2.4	.48	33	1.1	6.0	3.6
		--	7.4	3.6	6.2	59	83	6.0	.96	1.2	1.2	3.6	
		--	35	17	17	39	6.0	19	4.8	1.3	73	3.6	6.0
	C	1.4	1.2	23	1.2	70	2.4	8.4	9.6	8.4	.24	2.4	4.8
		--		29		110	64	18	4.8	8.4	1.2	2.4	4.8
		--	9.8	36	39	7.2	1.2	9.6	71	16	2.4		3.6
	D	36	17					90	190	52	48	65	4.8
		--	41	12	70	1.2	36	68	68			3.6	
	Field	9.0	.60	1.2								.60	4.3
Cladocera	A	--				580							
		--						610					
	B	--							290				510
		--	2.3			220	16		800			510	
		--					180				290		
	C						1000	450					
		--											
		--				160	35	290	290		160		1200
	D								17	16	480		
		--			450	1.6	740	810			130		
	Field												

EXPERIMENT IV

		day:	1	7	16	21	29	36	51	59
PHYTOPLANKTON $\frac{\mu^3}{mT} \times 10^5$			11/14/79							
Diatoms										
Stephanodiscus A			--	.98	2.6	.84	.13			
			--	1.3	.70	3.1	.18			
			--	1.8	2.0	6.2	3.1			
	B		--	1.6	4.2	.84	.13			
			--	.55	.84	1.6	3.1			
			--	2.0	1.1	1.6	.79			
	C		--	.98	.53	.088				
			--	1.6	1.4	.26	.090			
			--	.79	2.9	.79	3.9			
	D		--	1.4	4.0	3.1	.044			
			--	2.6	4.5	2.6	1.2			
			--	1.4	3.8	.088				
	Field	0'	.84	.97	4.1	2.6	2.2	3.7	1.4	.75
		12'	.40	2.4	3.8	3.8	2.4	1.6	1.3	.84
Asterionella	Field	0'				.060	.079	.060	.14	
	Field	12'					.11	.14	.071	.019
Fragilaria	Field	0'					.14	.61	.61	.14
	Field	12'				.35	.14	.070	.61	.14
Stephanodiscus II										
	Field	0'								1.0
	Field	12'								1.5

Flagellates									
Cryptochrysis	A	--	.17	.40	.046				
		--	1.1	.30	.015				
		--	.54	.28	.060				
	B	--	.34	.091	.015				
		--	.030		.015				
		--	.31						
	C	--	.71						
		--	.27	.023	.023				
		--	.20	.17					
	D	--	.44	.084					
		--	.30	.37	.091				
		--	.44	.33					
	Field 0'		.13	.12	.030	.036	.0075	.053	.0075
	Field 12'		.15	.22	.12	.0075	.038	.038	
Flagellate I	A	--			.0092				
	B,C,D	--							
	Field 0'				.028		.011	.064	
	Field 12'		.049			.014	.0028	.046	.0092
Flagellate II	A	--	4.7	6.5	6.4	5.3		4.2	
		--	2.9	11	17	.28		.44	
		--	.88	2.3	3.6	1.6		.53	
	B	--	2.9	2.4	2.6	9.0		.41	
		--	.88	2.8	4.6	1.5		.86	
		--	2.5	2.5	2.9	3.5		.061	
	C	--	4.6	6.5	6.7	7.4		--	
		--	1.9	9.6	20	6.7		1.3	
		--	3.0	21	5.9	.87		1.8	
	D	--	3.5	18	6.7	9.0		.053	
		--	2.1	3.4	1.7	1.3		.035	
		--	4.2	19	1.9				
	Field 0'			4.6					
	Field 12'								

Flagellate III	D	--						
		--						
		--				3.4		
Phacus	A	--						
		--						
		--						
	B	--						
		--	.45					
		--	.70					
	C	--						
		--						
		--						
	D	--						2.7
		--						.093
		--						.019
	Field 0'							
	Field 12'							
Others								
Staurastrum	A	--	.043	.0096				
		--						
		--	.043					
	B	--						
		--	.014					
		--						
	C	--						
		--						
		--						
	D	--						
		--		.019				
		--		.0095				
	Field 0'	.048	.029	.058	.077	.019	.030	.0096
	Field 12'	.067	.058	.091	.039	.014	.058	.0096
Ceratium	Field 0'							
	Field 12'		2.1					

Oscillatoria	A	--						.096	
		--							
		--							
	B	--							
		--							
		--							
	C	--				1.6		.45	
		--							
		--							
	D	--							
		--							
		--							
Anabaena sp.	C	--						.68	
		--							
		--							
NH_4^+ (μM)	A	6.3	3.5	2.3	2.8	1.6	2.6	3.3	2.9
			3.5	2.0	2.9	1.4	4.2	2.8	2.2
			9.3	5.4	6.0	2.9	3.1	2.7	2.2
	B		6.7	4.3	3.0	2.1	4.2	2.9	2.6
			7.2	4.1	2.8	2.7	6.2	4.4	4.2
			9.3	4.3	4.8	3.9	8.4	6.2	5.9
	C		7.4	2.3	2.7	1.6	2.3	3.0	2.6
			8.2	4.8	2.8	3.9	6.0	4.9	2.9
			7.5	2.5	2.4	3.2	4.5	3.7	3.3
	D		6.8	2.2	2.0	1.9	2.4	2.6	2.3
			7.6	3.0	2.8	2.1	2.9	3.0	2.5
			6.4	2.1	2.4	2.7	4.0	3.0	2.5
	Field 0'		5.8	--	2.7	2.6	5.3	4.2	3.7
	Field 12'	4.7	6.2	--	2.2	2.6	5.3	4.2	4.0

$NO_3^- + NO_2^-$ (μM)	A	4.0	2.8	1.8	7.0	2.0	2.1	2.3	2.2
			2.8	2.1	2.5	1.8	45.4	2.6	2.9
			3.7	3.1	2.8	3.0	3.7	3.2	1.9
	B		4.9	4.7	4.4	3.3	5.1	4.0	5.4
			2.7	2.9	2.8	3.1	3.4	5.0	4.4
			5.5	2.1	2.9	2.6	5.7	4.6	7.6
	C		4.1	2.2	3.0	1.9	2.8	8.3	2.5
			4.1	15.6	6.6	3.6	20.7	4.2	4.2
			4.1	11.5	5.2	2.9	3.7	4.0	4.0
	D		2.2	11.8	2.7	2.0	4.2	3.0	3.0
			6.5	4.3	6.8	2.8	2.9	2.8	2.5
			2.7	8.3	2.7	2.2	2.8	2.5	2.0
	Field 0'		3.6	--	1.7	2.5	3.4	8.2	5.8
	Field 12'	2.9	2.7	--	1.2	2.3	2.3	6.2	5.5

ZOOPLANKTON $\frac{\mu^3}{100\ ml} \times 10^6$								
Protozoa	A	--	.00070	.55	--		--	
		--	.0035	.29	--		--	
		--	.0014		--		--	
	B	--	.0021		--		--	
		--	.0091		--		--	
		--	.025		--		--	
	C	--	.034	.21	--	.25	--	
		--	.00070	.0048	--		--	
		--	.0077	.10	--		--	
	D	--	.0035	.026	--		--	
		--	.0084	.014	--		--	
		--	.0091	.022	--		--	
	Field 0'							
	Field 12'							

Rotifera	A	--	1.6	.51	1.7	.91	--	2.3	--
		--	.016	.29	.86	.15	--	.19	--
		--	.011	.38	.011	.39	--	.14	--
	B	--	1.6	.26	1.6	1.6	--	.57	--
		--	1.5	.11	.41	.32	--	1.7	--
		--	.048	.16		.094	--	.49	--
	C	--	.016		.69	.35	--	.053	--
		--	.080	.43	.34	.10	--	4.9	--
		--	.12	.19	.30	.096	--	.30	--
	D	--	.032	.080	2.1	1.5	--	.084	--
		--	1.5	.14	.33	1.4	--	9.3	--
		--	.088	.46	.34	.011	--	.065	--
	Field 0'	2.3	5.3		.016			.055	
	Field 12'	4.5	1.5	.75	2.3		.75	.040	.024
Copepoda	A	--	.12	6.3	.43	1.2	--	49	--
		--	8.7	3.5	2.3	5.3	--	14	--
		--	6.3	3.5	6.3	.12	--	19	--
	B	--		6.3			--	110	--
		--	10		6.8	1.6	--	17	--
		--	3.5	1.8		1.0	--	1.3	--
	C	--	6.9	.36	16	20	--	47	--
		--			18	1.5	--	61	--
		--	1.7	.24	2.5	7.8	--	1.3	--
	D	--	3.4	6.4	.96	14	--	40	--
		--		11	1.3	26	--	16	--
		--		.36	4.7	7.6	--	10	--
	Field 0'	5.4	18	.43	6.4		.060	2.0	.060
	Field 12'	19	7.9	.68	3.5		.49		2.4
Cladocera	A	--					--	4.0	--
		--				1.4	--	60	--
		--					--	20	--
	B	--					--	1.6	--
		--					--	1600	--
		--		1.6		.23	--	180	--
	C	--		1.7			--	89	--
		--					--	31	--
		--			51		--	290	--
	D	--					--	2.8	--
		--			.46	.92	--	37	--
		--		16		21	--	140	--
	Field 0'		130				8.0		
	Field 12'	8.0	220		15		26	83	

Section 8

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UNIVERSITY OF CALIFORNIA
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